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**Efectos de campos magnéticos que simulan tormentas
geomagnéticas en la presión arterial en ratas Wistar.**

TESIS

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DOCTORA EN CIENCIAS DE LA TIERRA**

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*Debes amar,
la arcilla que va en tus manos,
debes amar,
su arena hasta la locura
y si no,
no la emprendas
que será en vano.*

*Sólo el amor
alumbra lo que perdura,
sólo el amor
convierte en milagro el barro.*

*Debes amar,
el tiempo de los intentos,
debes amar,
la hora que nunca brilla
y si no
no pretendas tocar lo cierto.*

*Sólo el amor
engendra la maravilla,
sólo el amor
consigue encender lo muerto.*

*Sólo el amor
engendra la maravilla,
sólo el amor
consigue encender lo muerto.*

Fragmento de la canción "Sólo el amor" de Silvio Rodríguez

Gracias.....

*A ti mamá ...
que silenciosa guardas tus lágrimas para impulsar mis pasos...*

*A Juan...
sabia y cálida compañía....*

y a mis amigos de siempre ...

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*Y a quienes ya no están presentes pero que circulan en mi ser
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ESTRUCTURA DE LA TESIS:

La presente tesis se encuentra dentro de la modalidad de artículos publicados. Consta de:

- Introducción, en donde se presentan los estudios más recientes y relevantes que se han realizado a la fecha sobre efectos de tormentas geomagnéticas en el sistema cardiovascular, en particular en la presión arterial, y los posibles mecanismos fisiológicos que se han propuesto para explicarlos.

-Objetivos generales del trabajo.

- Tres artículos (dos publicados y uno aceptado) con sus respectivas presentaciones. Todos ellos en revistas del Índice de Citas.

- Una conclusión general.

INTRODUCCIÓN:

La presente investigación está dentro del campo de las Relaciones Sol-Tierra, se enfoca en los efectos de las tormentas geomagnéticas (TG) en seres vivos, particularmente en la presión arterial sistólica en mamíferos roedores, utilizando como modelo a la rata de laboratorio tipo Wistar.

La vida en nuestro planeta se desarrolló bajo variaciones de campo geomagnético, por lo que suponer que éstas tengan algún efecto en la fisiología de los seres vivos es una hipótesis plausible. Esta situación ha motivado a desarrollar estudios que proponen que los ritmos geomagnéticos influyen en diversos procesos fisiológicos y conductuales (Wever 1973; Cremer-Bartels et al., 1984; Funk et al., 2009, Stoupel et al., 2011, 2013; Shaposhnikov et al., 2014; Yu et al., 2014; Krylov et al., 2014).

Una línea de investigación se ha enfocado en la fisiología y morfología de la membrana celular como el lugar donde pueden observarse los efectos del campo magnético, en particular las alteraciones en la permeabilidad de iones o en la unión de ligando (es una molécula capaz de ser reconocida por otra provocando una respuesta biológica). Por ejemplo, un sitio receptor que actúa como modulador de cascadas en las que está implicado el calcio/calmodulina dependiente de procesos de señalización y factores de crecimiento (Markov 2011).

Otra línea de investigación se ha dirigido a la búsqueda de receptores a campos magnéticos, donde uno de los mecanismos físicamente viables es el de fotorreceptor de par-radical, (Ritz, et al., 2010; Johsen y Lohmann, 2008). El mecanismo fotorreceptor de par-radical es sensible al campo magnético externo debido a los efectos del campo magnético sobre los espines de los electrones separados en ambos radicales, que propone que el fotomagnetorreceptor contenga un pigmento que actúa como cofactor para transferir electrones al absorber la luz. Ritz et al. (2010) expone en su modelo, que un organismo es capaz de percibir variaciones del campo geomagnético del orden de un 2% si se tiene 10^8 pares de electrones en un volumen de 0.4 mm^3 .

El criptocromo es una proteína fotosensible y en particular el criptocromo 1^a (cry1A) se propone como candidata para ser magnetosensible por su capacidad de ser productora de pares de electrones además de absorber luz azul, otra cualidad importante es que se encuentra asociada a los fotorreceptores en la retina (Liedvogel et al 2007).

Otras investigaciones proponen que los campos magnéticos tienen efectos en los iones Ca^{2+} , donde la reactividad bioquímica de los iones unidos en las hendiduras moleculares de macromoléculas pueden verse afectados por los campos magnéticos estáticos, a través de los cambios en la orientación espacial del movimiento o cambiando las frecuencias de precesión de Larmor, con campos magnéticos del orden de nT (Muehsam y Pilla 1996).

Las variaciones de campo geomagnético provienen de diversas fuentes, en esta investigación nos concentramos en la asociada a TG. Hay estudios sobre el efecto del campo magnético débil sobre los sistemas biológicos que indican que las variaciones en la intensidad y la duración en el comienzo de una TG pueden alterar la dinámica a nivel

molecular en las transiciones de espín a través de mecanismos llamados magnetorrecepción a base de hierro-óxido (Ritz et al, 2010).

Muchas investigaciones asocian el comportamiento de las TG con respuestas fisiológicas tales como variación en la presión arterial o el aumento en la incidencia de muertes debido a enfermedades cardiacas como lo reportan Okano et al. (2008), Dimitrova et al. (2008), Khabarova and Dimitrova (2008), Mendoza and Sánchez de la Peña (2010; Kus´mina et al. (2014).

Los índices geomagnéticos reportan variación en el campo geomagnético asociado a TG y se han correlacionado con enfermedades cardiacas; por ejemplo las investigaciones Dimitrova et al. (2008), Shaposhnikov et al. (2014), Vencloviene et al. (2013), Mavromichalaki et al. (2012), Papailiou et al. (2011), Gurfinkel et al. (2012).

Hay reportes indicando que los campos magnéticos afectan la microcirculación arterial a través del baroreflejo iniciado por los receptores en la arteria arco aórtico, produciendo cambios de tono vasomotor periférico (Okano et al., 2008).

Estas investigaciones nos muestran que las variaciones de campo geomagnético producen respuesta fisiológica en sistemas biológicos. Cabe señalar que pocas de ellas resaltan los protocolos de control de las variables geomagnéticas y electromagnéticas ambientales.

Por lo que nuestras investigaciones incluyen un riguroso control de las mismas. Para ello se hizo un manejo de los animales involucrados de acuerdo a estándares internacionales y se usó una cámara semianecoica para apantallar el ruido electromagnético ambiental.

Nuestro estudio involucró a las TG ocurridas entre los años de 1996 al 2008 con $Dst \leq -100nT$ acompañado de un monitoreo de la actividad geomagnética durante los procedimientos experimentales, realizado por el Observatorio Geomagnético de Teoloyucan (TEO) que se encuentra relativamente cercano a la Facultas de Ciencias de la

UNAM, lugar donde se efectuaron los experimentos (TEO 19.74° N, -99 .18 W; UNAM 19.27°,-99.22W).

Hicimos un experimento en condiciones controladas cuyos resultados se reporta en los artículos que incluye esta tesis. Es importante hacer notar que no solo se logró un experimento sin contratiempos, sino que además ocurrió una TG con $Dst < -100nT$ en el periodo de experimentación y pudimos medir la presión arterial sistólica PA en las ratas. Observamos que la PA varió en un orden de 10% durante la TG, mientras era monitoreada en condiciones controladas. Además, por contar con los datos de componente horizontal del campo geomagnético (H) en tiempo real proporcionados por TEO, se pudieron reproducir los efectos con el uso de campos magnéticos simulados. Esta investigación produjo tres artículos que se presentarán a continuación tal y como serán publicados

1) Martínez-Bretón J.L., Mendoza B., Hernández-Quintero E. 2016. Relationship between the minima of the horizontal magnetic component measured in Mexico and the Dst and SYM-H indices for geomagnetic storms with $Dst \leq -100$ nT during the descending phase of solar cycle 23. *Geofísica Internacional* (2016) 55-2: 5-15.

2) Martínez-Bretón J.L., Mendoza B., 2015. Effects of magnetic fields produced by simulated and real geomagnetic storms on rats. *Advances in Space Research* 57 (2016) 1402-1410. doi : 10.1016/j.asr.2015.11.023.

3) Martínez-Bretón J.L., Mendoza B., Miranda-Anaya M., Durán P., Flores-Chávez P.L. 2016. Artificial Reproduction og Magnetic Fields Produced by a Natural Geomagnetic Storm Increases Systolic Blod Pressure in Rats. *International Journal of Biometeorology*. doi: 10.1007/s00484-016-1164-5. <http://link.springer.com/article/10.1007/s00484-016-1164-5>

La investigación se llevó a cabo en diferentes etapas.

1. Estudio de las TG ocurridas de 1996-2008 con $Dst < -100nT$, seleccionando 15 TG (2003-2005) con las que se construyó un perfil promedio del comportamiento de las TGs, conjuntamente se realizaron los programas necesarios para este efecto.
2. Fabricación de lo que denominamos un simulador (bobina, y programas de control) de variaciones de campo magnético que se alimentó con los datos de las variaciones magnéticas asociadas a la TG promedio, en las fases de comienzo súbito y fase principal. El simulador reprodujo la porción de la TG que produjo incrementos en la PA de las ratas durante el experimento.
3. Crianza y cuidado y condicionamiento pre-experimental de las ratas con las que se trabajó, así como la construcción de los implementos necesarios para los procedimientos experimentales, tales como cajas, luces LED rojas y blancas etc.
4. El experimento en sí, consiste brevemente en la estimulación de las ratas dentro de una Cámara Semianecoica para posteriormente medir la presión arterial por métodos no invasivos, con los respectivos controles.
5. Procesamiento de datos y escritura de artículos.

OBJETIVOS :

El objetivo general que conduce la investigación reportada en esta Tesis es determinar si las variaciones de campo magnético, similares en forma y magnitud a las presentadas en la H) del campo geomagnético al ocurrir una Tormenta Geomagnética (TG), en condiciones controladas, tiene efectos sobre la presión arterial sistólica en ratas adultas (Wistar).

Para lo cual se tuvieron que cumplir los siguientes objetivos particulares:

- a) Indagar el comportamiento de las TG con $Dst \leq -100nT$ entre los años de 1996-2008 a partir de la medición de H de TEO.
- b) A partir de los resultados obtenidos en el inciso a) construir un aparato que pudiera reproducir las variaciones tanto promedio, como locales reales de H, en un ambiente con blindaje electromagnético, la CS, así como implementos necesarios para la experimentación tales como luz LED, rejillas de materiales dieléctricos, cajas de transporte, etc.
- c) Contar con animales que estuvieran familiarizados con el experimentador así como con los procedimientos de experimentación.

El objetivo (a) se reporta en el artículo 1, los (b) y (c) se reportan en los artículos 2 y 3.

PRESENTACIÓN DEL ARTÍCULO: "*Relationship between the minima of the horizontal magnetic component measured in Mexico and the Dst and SYM-H indices for geomagnetic storms with $Dst \leq -100$ nT during the descending phase of solar cycle 23.*"

El diseñar un experimento con controles estrictos fue uno de los principales objetivos del trabajo. Al examinar investigaciones previas, notamos que una práctica frecuente era correlacionar directamente índices geomagnéticos tales como el Dst, con diferentes aspectos de la salud cardiovascular. Sin embargo la cuantificación de las diferencias locales con éstos índices no se abordaba.

Las diferencias locales son frecuentemente ocasionadas por corrientes ionosféricas, éstas pueden estar asociadas al lado día, noche, amanecer y anochecer, también muestran variaciones con las estaciones la latitud, y son afectadas por el ciclo solar.

Este primer artículo de la investigación doctoral cuantifica sus efectos, sin discernir específicamente cual fue la corriente en particular imperante al momento de ocurrir una TG. En el artículo se analizan las diferencias en aparición de mínimos entre los índices Dst, SYM-H y la H medida en TEO, observatorio ubicado en el Estado de México, con coordenadas geográficas (TEO 19.74° N, -99 .18 W), cuando ocurre una TG.

Las TGs que se analizaron fueron 15, éstas contaban con un registro claro de H medida por TEO y con un $Dst \leq -100$ nT entre los años de 2003 al 2005, correspondiente a la fase descendente del ciclo solar 23. En ellas se calculó la diferencia en tiempo universal en la

llegada al mínimo valor de Dst. Las discrepancias en tiempo las denominamos Δ y se muestra en el artículo como se obtuvieron.

Notamos que cuando TEO se encontraba en el lado día, amanecer y atardecer, la Δ fue negativa indicado que el mínimo apareció por primera vez en el Dst y SYM-H reportado por Kyoto (http://wdc.kugi.kyoto-u.ac.jp/dst_provisional/index.html) y después en H medido por TEO (<http://www.intermagnet.org/index-eng.php>). Por otra parte, cuando TEO se encontraba cercano a la medianoche la diferencia fue positiva, lo que indica que el mínimo se reportó antes en TEO y después en Dst. Notamos que 14 de las 15 tormentas geomagnéticas siguieron este comportamiento, excepto la más intensa de la muestra. Para las 14 restantes el tiempo de desfaseamiento en los mínimos no parece depender de la intensidad de la tormenta geomagnética, sino de la intensidad de los sistemas de corrientes presentes.

Relationship between the minima of the horizontal magnetic component measured in Mexico and the Dst and SYM-H indices for geomagnetic storms with $Dst \leq -100nT$ during the descending phase of solar cycle 23

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Resumen

En este trabajo se analizan las diferencias en aparición de mínimos entre los índices Dst, SYM-H y la componente horizontal H medida en el Observatorio Magnético Teoloyucan (TEO) ubicado en México. Se calculó esta diferencia en tiempo universal para 15 tormentas geomagnéticas ($Dst \leq -100nT$) que ocurrieron durante la fase descendente del ciclo solar 23. Notamos que cuando TEO se encontraba en el lado día, amanecer y atardecer, la diferencia horaria fue negativa, indicando que el mínimo apareció por primera vez en el Dst y SYM-H reportado por Kyoto y después en H medido por TEO. Por otra parte, cuando TEO se encontraba cercano a la medianoche la diferencia es positiva, lo que indica que el mínimo se reportó antes en TEO y después en Dst. Notamos que 14 de las 15 tormentas geomagnéticas siguieron este comportamiento, excepto la más intensa de la muestra. Para las 14 restantes el tiempo de desfase en los mínimos no parece depender de la intensidad de la tormenta geomagnética, sino de la intensidad de los sistemas de corrientes presentes.

Palabras clave: corrientes de la Ionosfera, DST, SYM-H, tormentas geomagnéticas intensas, relaciones solar-terrestres.

Abstract

In this paper, we analyze the time delay between the occurrence of the minima in the geomagnetic Dst, SYM-H indices and the horizontal magnetic component (H) measured in the Teoloyucan Magnetic Observatory (TEO) of Mexico. This difference was calculated in Universal Time for 15 geomagnetic storms ($Dst \leq -100nT$) occurred during the descending phase of solar cycle 23. We found that, when the TEO was at the dayside, dawn and dusk, the time difference was negative, indicating that the minimum appeared first in the Dst, SYM-H reported by Kyoto, and afterwards in the H reported by TEO. On the other hand, when the TEO was close to midnight the difference was positive, indicating that the minimum occurred first at TEO and afterwards in Dst. We noticed that 14 out of 15 geomagnetic storms followed this behavior, except the most intense one of the sample. For the rest of the storms, it seems that the cause of the delay is not the intensity of the magnetic field at minimum but the intensity of the current systems present during the storm occurrences.

Key words: ionosphere currents, Dst, SYM-H, intense geomagnetic storms, solar-terrestrial relations.

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Introduction

Geomagnetic activity in general and geomagnetic storms (GS) in particular, are manifested through a series of processes involving current systems that induce magnetic fields, measured by several geomagnetic ground observatories.

The intensity of the GS is given by the geomagnetic Dst (Disturbance storm time) (Sugiura, 1964) index. During the GS, there are several current systems at play. The storm starts with the momentum and plasma transfer from the solar wind to the magnetosphere (Dessler and Parker, 1959), which produces an intensification of the magnetopause currents, the field aligned currents and the ring currents; the latter produce the characteristic decrease in the Dst index. There is also the partial ring current that is one of the current systems located in the Northern and Southern Hemispheres at the geomagnetic equatorial plane, and closed through the ionospheric field aligned currents (Kalegaev, *et al.*, 2008; Li, *et al.*, 2011; Lockwood, 2013) and the magnetotail current system (Alexeev, *et al.*, 1996). The intensity of each current system is a consequence of the energy injected to the magnetosphere by the solar wind (Clúa *et al.*, 2013; Patra *et al.*, 2011).

The Dst index was created 50 years ago (Hamilton *et al.*, 1988), in order to have a quantitative measurement of the ring current. Four observatories monitor the Dst: Honolulu (Longitude (E) 201.98°, Latitude 21.32°), San Juan (Longitude (E) 293.88°, Latitude 18.11°), Hermanus (Longitude (E) 19.22°, Latitude -34.40°) and Kakioka (Longitude (E) 140.18°, Latitude 36.23°) located at mid and low latitudes (see Figure 1) where the influence of the equatorial electrojet is minimum. Each observatory reports the horizontal magnetic field intensity (H), and the Dst is constructed from these four reports. The Dst minimum indicates the moment of occurrence of the GS in universal time (UT) (Mandea and Korte, 2011; Campbell 2004; Mayaud 1980; Sugiura, 1964).

The SYM-H index describes the geomagnetic disturbance field at mid-latitudes with a 1 min resolution. Dst and SYM-H are argued to be essentially the same (e.g., Sugiura and Poros, 1971) as their numerical differences are small. However, a recent work (Katus and Liemhon, 2013) shows that, although their correlation is 0.9, they are inherently different, because each index applies different methods to remove irrelevant fluctuations.

The derivation procedure for both the Dst and the SYM-H essentially consists for the following four steps: (1) Subtraction of the geomagnetic main field and the Sq (solar quiet daily variation) field to calculate the disturbance field component. (2) Coordinate transformation to a dipole coordinate system. (3) Calculation of the longitudinally symmetric component (i.e. six-station average for SYM-H and four stations average for Dst) and the asymmetric component (i.e. disturbance field minus the symmetric component). (4) Derivation of the asymmetric indices (i.e. the range between the maximum and the minimum asymmetric fields), <http://wdc.kugi.kyoto-u.ac.jp/aeasy/asy.pdf>

As a first approximation, the ring current is a toroidal westward current system centered at the equatorial plane, at a geocentric distance between 2 and 9 terrestrial radii (Bogdanova *et al.*, 2014). It is formed mainly by positive ions and ~25% electrons (Liu *et al.*, 2005), with energies between tens and hundreds keV and subjected to an azimuthal drift. The ring current increases its density during the GS main phase, decreasing during the recovery phase. The loss mechanisms are more efficient near dawn and dusk (Le, 2013), which explains why the ring current and the ionosphere control the electric fields in the interior of the magnetosphere at dawn and dusk (Bogdanova *et al.*, 2014). These effects are due to the electric fields that appear during the dusk near the equatorial ionosphere (Tsurutani *et al.*, 2012). The magnetotail currents are also systems that strongly contribute to the Dst decrease during a GS. The ring current is very important for the ionosphere/magnetosphere dynamics (Hamilton *et al.*, 1988). It has been shown that the O⁺ ions, of ionospheric origin, contribute significantly to the plasma pressure of the inner magnetosphere during a GS (Keika *et al.*, 2013; Daglis *et al.*, 1999; Welling *et al.*, 2011). Also, strong ionospheric effects associated with a GS have been reported. The ionospheric local currents affect the H, thus the measurements of the geomagnetic observatories depend on the latitude, and the Magnetic Local Time (MLT) (Shinbori *et al.*, 2012; Ahn *et al.*, 2002).

There is a strong dependence of the ionospheric conductivity and the decrease of H. Also, there is a seasonal dependence of the sudden storm commencement amplitude and the MLT, according to the current systems involved (Shinbori *et al.*, 2012).

The purpose of the present work was to determine, and evaluate the difference in time during a GS between the occurrence of

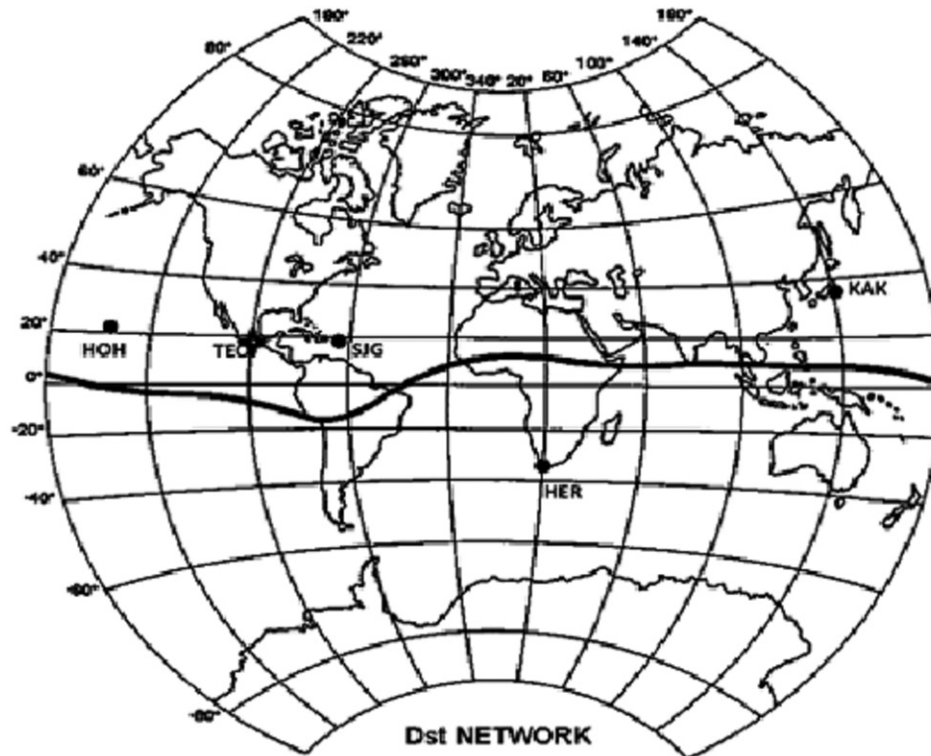


Figure 1. Map showing the location with circle of the four stations that are used to construct the Dst index: Honolulu (Longitude (E) 201.98°, Latitude 21.32°), San Juan (Longitude (E) 293.88°, Latitude 18.11°), Hermanus (Longitude (E) 19.22°, Latitude -34.40°) and Kakioka (Longitude (E) 140.18°, Latitude 36.23°). The star indicates the location of the Teoloyucan Magnetic Observatory (Longitude (E) 260.81°, Latitude 19.74°) (<http://wdc.kugi.kyoto-u.ac.jp/dst/dir/dst2/onDstindex.html>).

the minima in Dst, SYM-H and H measured in the Teoloyucan Magnetic Observatory (TEO) of Mexico. Knowing these differences is important for research concerning local geomagnetic phenomena.

Methods

TEO is located in Mexico at Longitude (E) 260.81°, Latitude 19.74° (see Figure 1). The vector of the magnetic field has been continuously monitoring since 1914, being one in a worldwide network of magnetic observatories and belonging to the international INTERMAGNET project. Besides, TEO is the backbone of the Magnetic Service of the Geophysics Institute of the National Autonomous University of Mexico (Instituto de Geofísica de la Universidad Nacional Autónoma de México) and the hub of a broad range of geophysical and magnetic field research in Mexico.

The data base we use corresponds to the TEO H component (TEO-H) from January 2003 to December 2006 corresponding to the descending phase of solar cycle 23 (<http://>

www.intermagnet.org/data-donnee/download-eng.php and <http://geomaglinux.geofisica.unam.mx/>). We also used the Dst data for GS with $Dst \leq -100$ nT (There are reports of effects on biological systems) from the World Data Center for Geomagnetism Kyoto, (<http://wdc.kugi.kyoto-z.ac.jp/index.html>) for the same time period. We processed 113 976 Kyoto hourly data, and 1,721,820 TEO-H and SYM-H data reported each minute. For SYM-H the data were taken from (http://omniweb.gsfc.nasa.gov/form/omni_min.html).

To calculate the time differences (Δ) between the GS minima reported by the Dst and the TEO-H, we proceeded as follows.

We located the TEO-H data within a window of two days, before and three days after the day of the Dst minimum. We identified 15 GS within this window with $Dst \leq -100$ nT.

They were organized according to the time of occurrence in UT; then TEO-H local time was calculated for each GS (Table 1).

Table 1. The 15 geomagnetic storm with $Dst \leq -100nT$ corresponding to solar cycle 23.

| Event | Date | UT hours | TEO MLT hours | Dst minimum nT | Δ minutes | Δ_{SYM-H} |
|-------|------------|----------|---------------|----------------|------------------|------------------|
| GS 1 | 04/04/2004 | 1:00 | 19:00 | -117 | -23 | -1 |
| GS 2 | 30/08/2004 | 23:00 | 17:00 | -129 | -25 | -3 |
| GS 3 | 20/11/2003 | 22:00 | 16:00 | -422 | 114 | -2 |
| GS 4 | 31/08/2005 | 20:00 | 14:00 | -122 | -122 | -153 |
| GS 5 | 08/05/2005 | 19:00 | 13:00 | -110 | -87 | -1 |
| GS 6 | 18/08/2003 | 16:00 | 10:00 | -148 | -176 | -472 |
| GS 7 | 22/01/2004 | 14:00 | 08:00 | -130 | -385 | -636 |
| GS 8 | 27/07/2004 | 14:00 | 08:00 | -170 | -94 | -82 |
| GS 9 | 30/05/2005 | 14:00 | 08:00 | -113 | -240 | -126 |
| GS 10 | 11/09/2005 | 11:00 | 05:00 | -139 | -179 | -215 |
| GS 11 | 18/06/2003 | 10:00 | 04:00 | -141 | -63 | -60 |
| GS 12 | 18/01/2005 | 9:00 | 03:00 | -103 | 52 | 312 |
| GS 13 | 15/05/2005 | 9:00 | 03:00 | -247 | 14 | 1 |
| GS 14 | 08/11/2004 | 7:00 | 01:00 | -374 | 116 | 84 |
| GS 15 | 12/07/2003 | 6:00 | 00:00 | -105 | 234 | 262 |

Δ = time difference between the Dst minimum and the TEO-H minimum occurrence; Δ_{SYM-H} = time difference between the SYM-H minimum and the TEO-H minimum occurrence.

Besides, as the other hand, as the geomagnetic index Dst is hourly, it was necessary to carry out an interpolation with resolution of one minute. In order to do this, Hermite interpolating polynomials were used the interpolation was performed based on a group of data pairs $\{(t_1, f(t_1)), (t_2, f(t_2)), \dots, (t_k, f(t_k)), \dots\}$. Two successive values of time t_k differ in one hour, that is $t_{k+1} - t_k = 1h$, where $f(t_k)$ is the data that corresponds to the time t_k . The interpolating polynomial $P_k(t)$ was obtained starting from the points $\{(t_k, f(t_k)), (t_{k+1}, f(t_{k+1}))\}$. These polynomials are built in such a way that the derivative is continuous in the node points $(t_k, f(t_k))$, that is to say, two adjacent interpolating polynomials $P_k(t)$ and $P_{k+1}(t)$ are smoothly united. This limits the function formed by the union of the polynomials $P_k(t)$ in such a way that it does not become too disperse, and allows it to present less variations if the data are not smooth.

Once we located the minimum for each of the obtained functions, related to each GS (using either the Dst_{min} or SYM-H), we calculated Δ and Δ_{SYM-H} according to the following expressions (1a) and (1b):

$$\Delta = Dst_{min} - TEO-H \quad (1a)$$

or

$$\Delta_{SYM-H} = SYM-H - TEO-H \quad (1b)$$

Δ differences are given in minutes.

Results and discussion

In order to distinguish relevant magnetospheric current systems, we have sketched several figures. They represent the Earth from the North Pole; the inner circle represents the universal time (UT) and the outer circle represents the MLT.

Thick lines are at the night side and thin lines at the day side. Regions of currents are: the magnetotail electrojet in the night side, the dawn, the dusk and the day side.

To obtain Δ and Δ_{SYM-H} we compare directly the plots of Dst or SYM-H and TEO-H. According to expressions 1a or 1b, a negative (positive) Δ or Δ_{SYM-H} means that the minimum appears first (afterwards) in the Dst or SYM-H indices (Dst_{min} or $SYM-H_{min}$) and afterwards (first) in

the TEO-H. Figure 2 shows GS 1, GS 2 and 4 to 11, and Table 1 indicates that, for these storms, Δ or Δ_{SYM-H} is negative. Figure 3 shows GS 12 to 15, and Table 1 shows that for these storms Δ is positive. Figure 4 presents GS 3, as Table 1 indicates that it has a positive Δ or Δ_{SYM-H} . In the next section, we will discuss in the next section this storm in particular.

Figures 5, 6 and 7 show the plots of GS 6, 12 and 3 respectively. The GS_6 (Figure 5) is an example of a GS, with negative Δ , and occurred on the 18th of August 2003 with Dst = -148nT. The GS_12 in Figure 6 is an example of a storm with positive Δ , occurred on the 18th of January 2005 with Dst = -103nT. Finally, GS_3 Figure 4 occurred on the 20th of November, 2003 with Dst = -422 nT, being the most intense of the whole sample.

Figure 2 and Table 1 show that all TEO-H minima that occur in the day side, dawn and dusk have a negative Δ (GS 1, GS 2 and 4 to 11), except GS 3, which has positive Δ . Figure 3 shows that all TEO-H minima that occur in the night side have a positive Δ (GS 12 to 15).

From Figure 2, and Δ , we see that when the TEO is under the influence of the ionospheric currents associated to the day side, down

and dusk, the minimum occurs first in the Dst minimum and afterwards in TEO, so Δ is negative. Furthermore, we notice that GS 2, 4, 5 and 6 are influenced by the equatorial electrojet, which is very strong during the daytime [Kalegaev *et al.*, 2008]. Also, in the day side dawn and/or dusk, the GS 7, 8 and 9, occurred at the same time in different dates, that is 14:00UT and in local time at 8:00hrs in the morning. The values of the deltas do not seem to depend on the intensity of the GS expressed in the value of Dst, hence we assume that they are caused by ionospheric currents.

The GS 10 presents a minimum in TEO at 5:00 MLT, then it is under the influence of the dawn current systems. For GS 11 (Figure 2) and 12 (Figure 3), according to Table 1, we notice a change of sign in Δ ; we propose that this happens because they occur in the border of influence of the dawn currents.

These results agree with Li *et al.* (2011), who found that during the ring current injection of a magnetic storm, ions are mostly present in the dusk and pre-midnight sectors of the Earth, because of duskward drifting, producing a highly asymmetric geomagnetic disturbance with MLT.

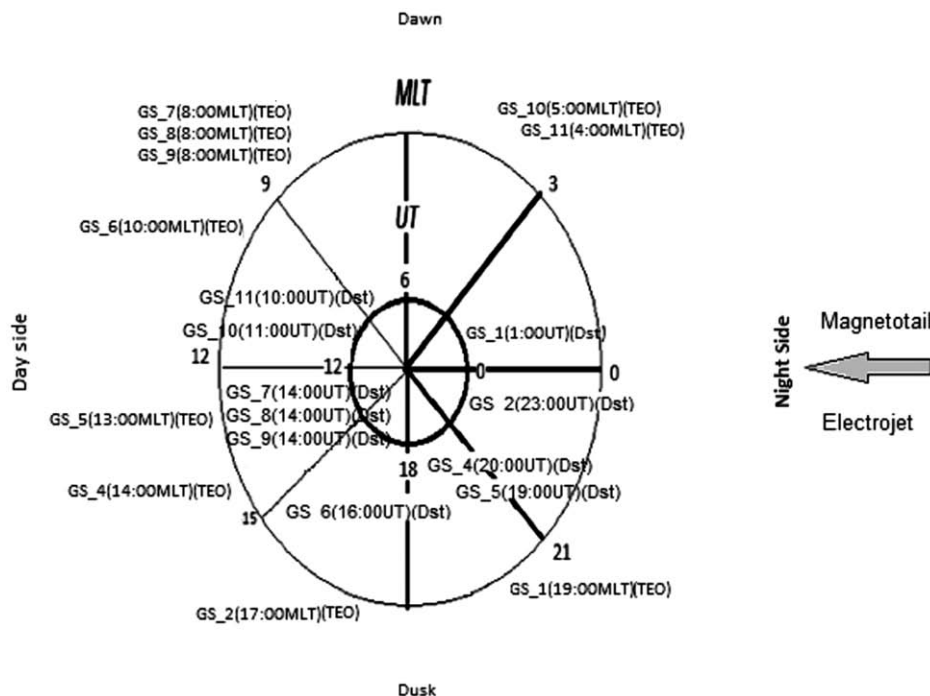


Figure 2. The Earth as seen from the North Pole. The inner circle corresponds to Universal Time (UT) and the outer circle to Magnetic Local Time (MLT). Also are shown the locations of geomagnetic storms (GS) at the moment of occurrence of the storms. The figure shows those GS with negative Δ (GS 1, GS 2 and 4 to 11 according to Table 1).

The GS 12 and 13 have positive Δ and Δ_{SYM-H} (Figure 3 and Table 1), and are located in the zones of the night side current systems. The GS 14 and 15 are located near the magnetotail electrojet zone. In these cases, the electrojet injects directly the ring current when TEO is nearby it, recording first the minimum in TEO-H.

Finally, we noticed that the magnitude of Δ and Δ_{SYM-H} is not related to the GS intensity for all the GS studied except GS 3 (Table 1). Concerning GS 3, we propose that somehow its great intensity (Dst = -422nT) would increase the complexity of all the involved phenomena. Also, the difference in Δ would depend on the intensity of the ionospheric currents that are present at the moment of the GS. Then, it seems that these currents would be capable of locally delay or forward the appearance of the minimum in TEO.

According to Cid *et al.*, (2014) GS have a significant local variation, which agrees with GS 14 and 15, that present large Dst minima. However GS_3, having a Dst= -422nT, has a very small local variation.

It has been argued that the presences of currents affect the local measurements of the geomagnetic observatories [Shinbori *et al.*, 2012]. Knowing the difference in time for the

occurrence of the minima in Dst and local is important to better quantify the local impacts of GS, that is why a direct correlation between local measurements and Dst should be analyzed.

Conclusions

We analyzed the differences in time (Δ and Δ_{SYM-H}) between the occurrence of the minima in Dst, Δ_{SYM-H} and TEO-H, calculated in UT for 15 GS, Dst \leq -100nT, during the descending phase of the solar cycle 23 (January 2003 to December 2006).

We found that when TEO is in the day side, dawn or dusk, the minimum appears first in Dst and afterwards in TEO (negative Δ , Δ_{SYM-H}). However, GS 3 did not follow this behavior, perhaps due to its great intensity; however we cannot explain yet why. We suppose that the day side, dawn and dusk ionospheric currents are the reason of the delay. When TEO is near midnight the minimum is found first in TEO and afterwards in Dst (positive Δ , Δ_{SYM-H}); again we suppose that the magnetotail currents are the cause of this delay.

The differences between Sym-H and Dst, may be because one is an average time and Sym-H is per minute, and that data can come from different observatories.

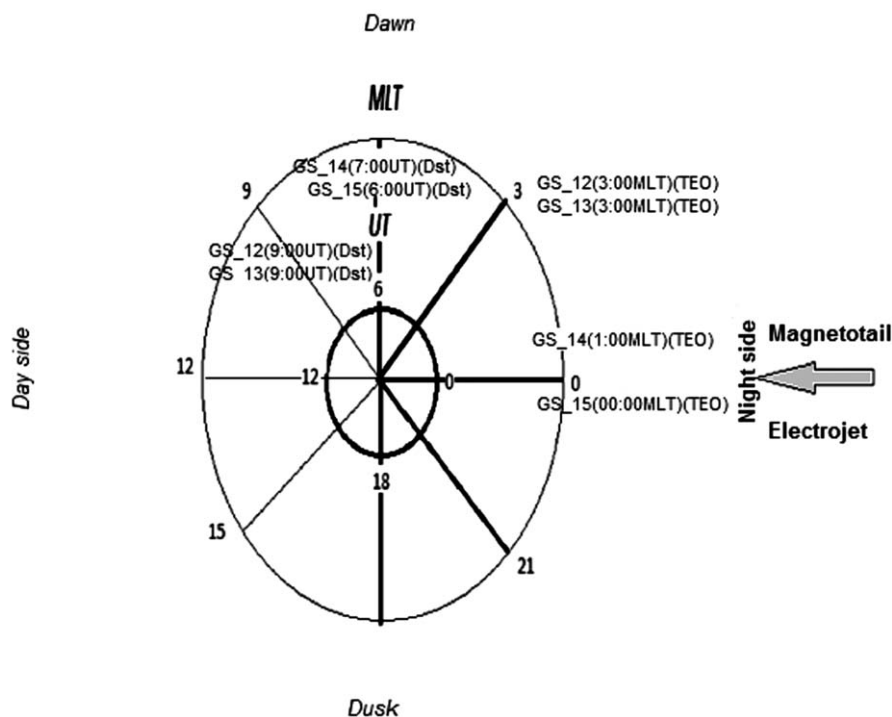


Figure 3. As Figure 2 but showing those GS with positive Δ (GS 12 to 15 according to Table 1).

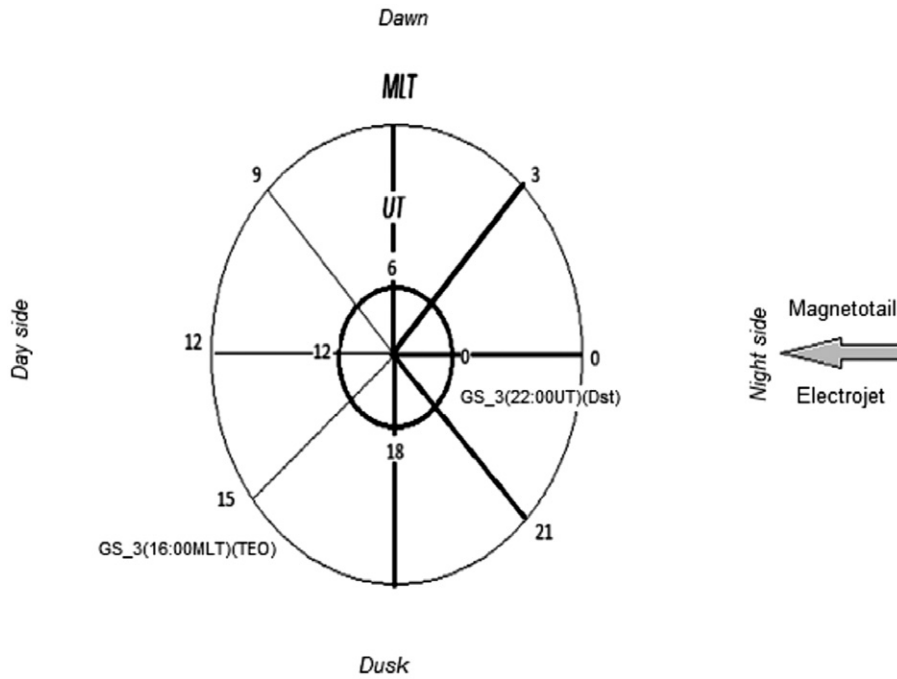


Figure 4. As Figure 2 but showing the location of the GS 3 (according to Table 1) at the moment of occurrence of the storm.

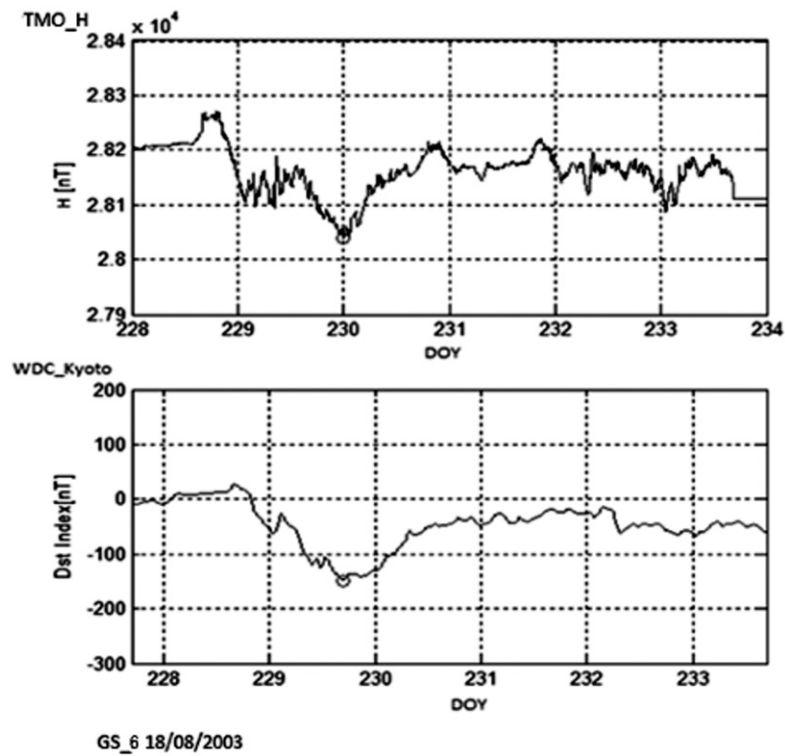


Figure 5. Example of a storm with a negative Δ . GS 6 (see Table 1) shows at the top the TEO-H and at the bottom the Dst. The small empty circle marks the minimum. The minimum presents first in Dst and afterwards in TEO-H.

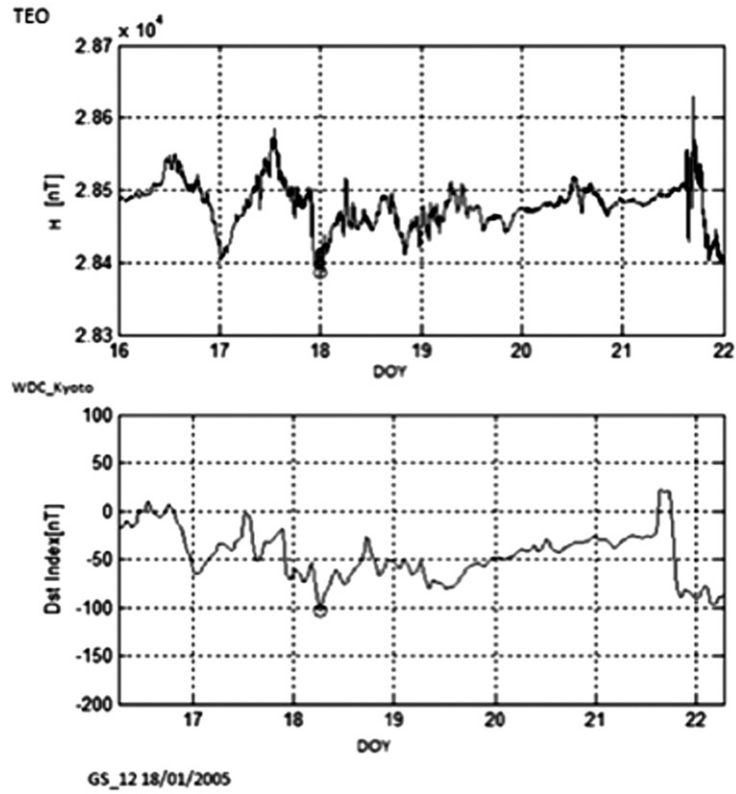


Figure 6. Example of a storm with a positive Δ . GS 12 (see Table 1) shows at the top the TEO-H and at the bottom the Dst. The small empty circle marks the minimum. The minimum presents first in TEO-H and afterwards in Dst.

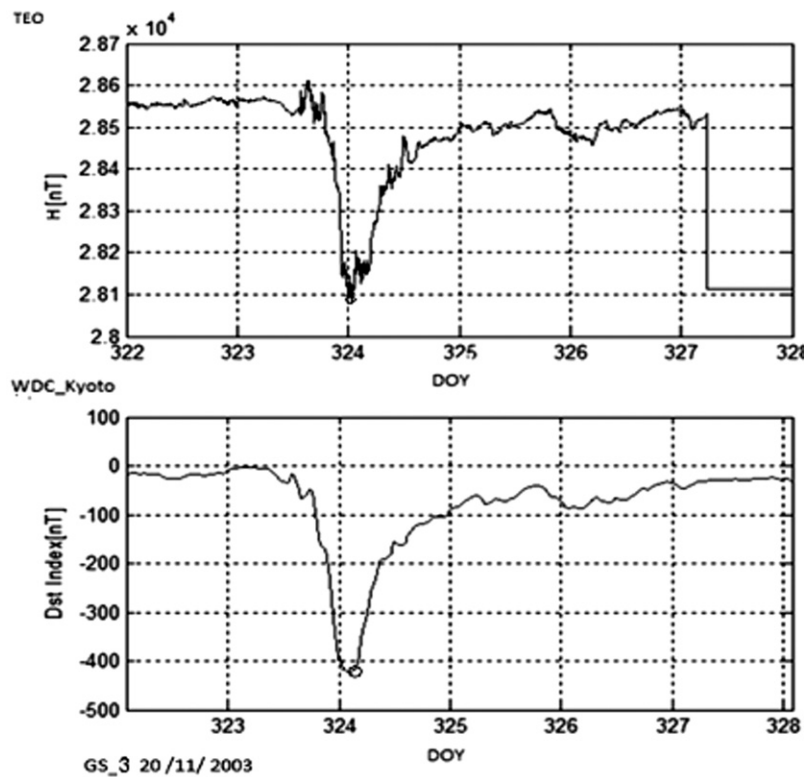


Figure 7. GS 3 (see Table 1) presents a positive Δ . At the top is the TEO-H and at the bottom the Dst. The small empty circle marks the minimum. The minimum presents first in TEO-H and afterwards in Dst. This storm has the largest intensity of the sample (Dst=-422nT).

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PRESENTACIÓN DEL ARTÍCULO: "*Effects of magnetic fields produced by simulated and real geomagnetic storms on rats.*"

El artículo anterior indica que hay que considerar las corrientes locales que pueden generar variaciones en las lecturas de H registradas por TEO durante todo el periodo experimental. Por lo que se contó con un registro continuo de H durante este periodo realizado por TEO, además se estuvo atento de la actividad solar que pudiera generar una TG, la cual ocurrió. Este segundo artículo se centra en los aspectos geomagnéticos y de instrumentación relevantes previos y durante el periodo experimental. Aquí mostramos como se realizaron las estimulaciones de campo magnético. Por un lado las que corresponden a variaciones de campo magnético del tipo de las que se producen durante el Dst asociadas a TGs con $Dst \leq -100nT$. Para ello se realizó una búsqueda en las bases de datos proporcionados por Kyoto, Omni Web y TEO, entre los años de 1996 al 2008, dentro del ciclo solar 23. Se encontraron 15 TGs cuyos registros en TEO estuvieran completos. A partir de éstas se construyó con el uso de MtLab un perfil promedio separando las fases de comienzo súbito (SSC) y fase principal (PF) que serían reproducibles con un simulador construido para tal efecto.

El Simulador es capaz de reproducir estas variaciones de campo magnético por medio de una bobina así como de un circuito que controlaba el paso de la corriente para reproducir la variación del campo magnético deseada. El circuito consta de un convertidor digital analógico de 12 bits y de un Arduino Due.

Contamos además con una cámara semianecoica, la cual permite el paso del campo geomagnético sin embargo nos ofrece un blindaje electromagnético ambiental para campos magnéticos de hasta 10^{-8} T.

Durante el periodo de experimentación ocurrió una TG y pudimos reproducir las variaciones de la componente H reportadas por TEO en el simulador que reprodujo las estimulaciones de variaciones de campo magnético.



Effects of magnetic fields produced by simulated and real geomagnetic storms on rats

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Abstract

In this paper we report experiments of arterial pressure (AP) measurements of ten Wistar rats subjected to geomagnetic field changes and to artificially stimulated magnetic field variations. Environmental electromagnetic effects were screened using a semianechoic chamber, which allowed us to discern the effects associated with geomagnetic storms. We stimulated the subjects with a linear magnetic profile constructed from the average changes of sudden storm commencement (SSC) and principal phases of geomagnetic storms measured between 1996 and 2008 with $Dst \leq -100$ nT. Although we found no statistically significant AP variations, statistically significant AP changes were found when a geomagnetic storm occurred during the experimental period. Using the observed geomagnetic storm variations to construct a geomagnetic profile to stimulate the rats, we found that the geomagnetic field variations associated to the SSC day were capable of increasing the subjects AP between 7% and 9% from the reference value. Under this magnetic variation, the subjects presented a notably restless behavior not seen under other conditions. We conclude that even very small changes in the geomagnetic field associated with a geomagnetic storm can produce a measurable and reproducible physiological response.

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Keywords: Geomagnetic storms; Simulated geomagnetic storms; Arterial pressure in rats

1. Introduction

Geomagnetic storms (GS) affect space-borne or ground-based technological systems and may even affect living organisms, and in particular, the human cardiovascular systems (Vencloviene et al., 2013).

The Dst index is frequently used as an indication of the GS relative strength (Gonzalez et al., 1994; Hamilton et al., 1988; Spencer et al., 2013; Kalegaev et al., 2015). The GS's are categorized as weak ($Dst < -30$ nT), moderate ($Dst < -50$ nT), strong ($Dst < -100$ nT), severe

($Dst < -200$ nT) and great ($Dst < -350$ nT) (Olawepo and Adeniyi, 2014).

It has been observed that the Dst associated to a GS typically shows a three-phase pattern: a sudden storm commencement (SSC), followed by a period of fast decay or principal phase (PF) and finally a recovery phase (RF) where gradually the Dst returns to its quiet time value. Although this is the canonical behavior, the SSC can be present or not (Takahashi et al., 1991; Feldstein et al., 2000; Spencer et al., 2011) (see Fig. 1).

Life on Earth has evolved in the presence of natural magnetic fields everywhere, and several studies indicate that biological systems respond to a wide range of magnetic field intensities (e.g. Zhang et al., 2014; Khabarova and Dimitrova, 2009; Krylov et al., 2014; Yu and Shang, 2014). There are an important number of papers concern-

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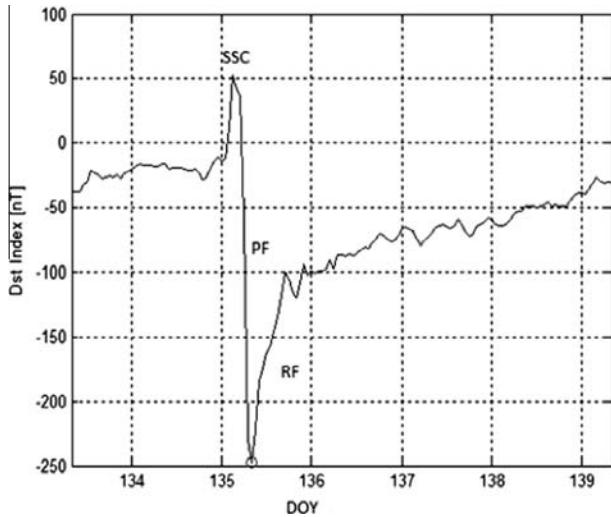


Fig. 1. The three phases of a geomagnetic storm through the Dst index: the sudden storm commencement (SSC), the principal (PF) and recovery (RF) phases. The DOY corresponds to day of the Year.

ing geomagnetic activity and human health. Recent studies dealing with such problem are for instance: Shaposhnikov et al. (2014), who correlated meteorological and geomagnetic factors with myocardial infarctions and brain strokes; Vencloviene et al. (2013), who correlated solar activity and meteorological variables with myocardial infarctions and cardiac health; Mavromichalaki et al. (2012), who found a correlation between geomagnetic activity and cardiac health; Gurfinkel et al. (2012), correlated temperature and geomagnetic activity with vascular parameters; Papailiou et al. (2011), using data from aviators, showed a significant correlation between heart rate variations and high levels of geomagnetic activity and strong cosmic ray intensity decreases; Stoupelet al. (2011), after a twenty year study reported variations in physiological parameters associated with cosmic rays, solar and geomagnetic activity, in particular the finding that cardiovascular health was affected; Mendoza and Sánchez de la Pena (2010), reviewed many papers finding correlations between geomagnetic activity and human health at middle and low geomagnetic latitudes; Khabarova and Dimitrova (2009), found an influence of environmental parameters such as atmospheric pressure, temperature or geomagnetic activity on cardiovascular health; and Dimitrova et al. (2009), found a good correlation between increases in systolic and diastolic pressure and a significant increase in geomagnetic activity.

Furthermore, there are also studies reporting specifically arterial pressure changes during the day before a GS, a time including a SSC: Dimitrova et al. (2004) found that participants presented pressure increases under weak local geomagnetic changes and when major and severe global geomagnetic storms took place. Dimitrova et al. (2009) reported an increase in systolic and diastolic pressure.

In the present paper, we paid special attention in separating the effects of the geomagnetic field variations from the ambient magnetic field variations on physiological

processes. Moreover, we identified which phase of the GS caused the largest physiological responses.

2. Geomagnetic storm indices

2.1. Data

For the derivation of the Dst index, four magnetic observatories, Hermanus, Kakioka, Honolulu, and San Juan are used. These observatories were chosen on the basis of the quality of observations, that their locations are sufficiently distant from the auroral and equatorial electrojets and that they are distributed in longitude as evenly as possible (<http://wdc.kugi.kyoto-u.ac.jp/dstdir/dst2/onDstindex.html>). The Dst index is the hourly average of the data. There is also the SYM-H index that has a one-minute resolution and is constructed similarly to the Dst. It calculates the symmetric portion of the magnetic field horizontal component (H) near the equator (Wanliss and Showalter, 2006).

To assess the GS behavior we used the Dst and SYM-H indices and the H data. The indices are found in the Space Physics Data Facility OMNIWeb (<http://omniweb.gsfc.nasa.gov/form/dx1.html>) and the WDC_Kyoto (<http://wdc.kugi.kyoto-u.ac.jp/index.html>). The H data was obtained from the Teoloyucan Magnetic Observatory (TEO) (<http://geomaglinux.geofisica.unam.mx/>) INTERMAGNET network (<http://www.intermagnet.org/data-donnee/download-eng.php>), respectively. The TEO observatory has the following geographic coordinates: latitude 19.75° N, longitude -99.17° W.

2.2. Methodology

We used two time periods: the first one from January 1996 to December 2008 and the second one from the 4th of February to the 10th of April 2014. Fig. 2a shows the Dst behavior of the WDC_Kyoto data during the latter period.

The first period data was used to construct a mean GS profile. We searched for strong GS ($Dst < -100$ nT) in the three databases from 1996 to the end of 2008, finding 15 GS registered in the three observatories. They appear in Table 1. We obtained the SSC and PF phase's means of the 15 GS, and constructed two profiles named eSSC and ePF, respectively shown in Fig. 3a and b. During the second period of the experiment, there was only one GS with $Dst < -100$ nT. It did not present a SSC as such, but a gradual increase of the Dst during approximately two hours (see Fig. 2b) the 18th of February 2014. The PF minimum the 19th of February 2014 at 9:00 UT with $Dst = -112$ nT, and the RF persisted up to the 27th of February, 2014. We used the 18th of February data (the gradual increase data), subtracting the H average for that day ($H = 27611.9$ nT) to obtain the variations with respect to this average, to construct a profile named Profile_GS shown in Fig. 3c.

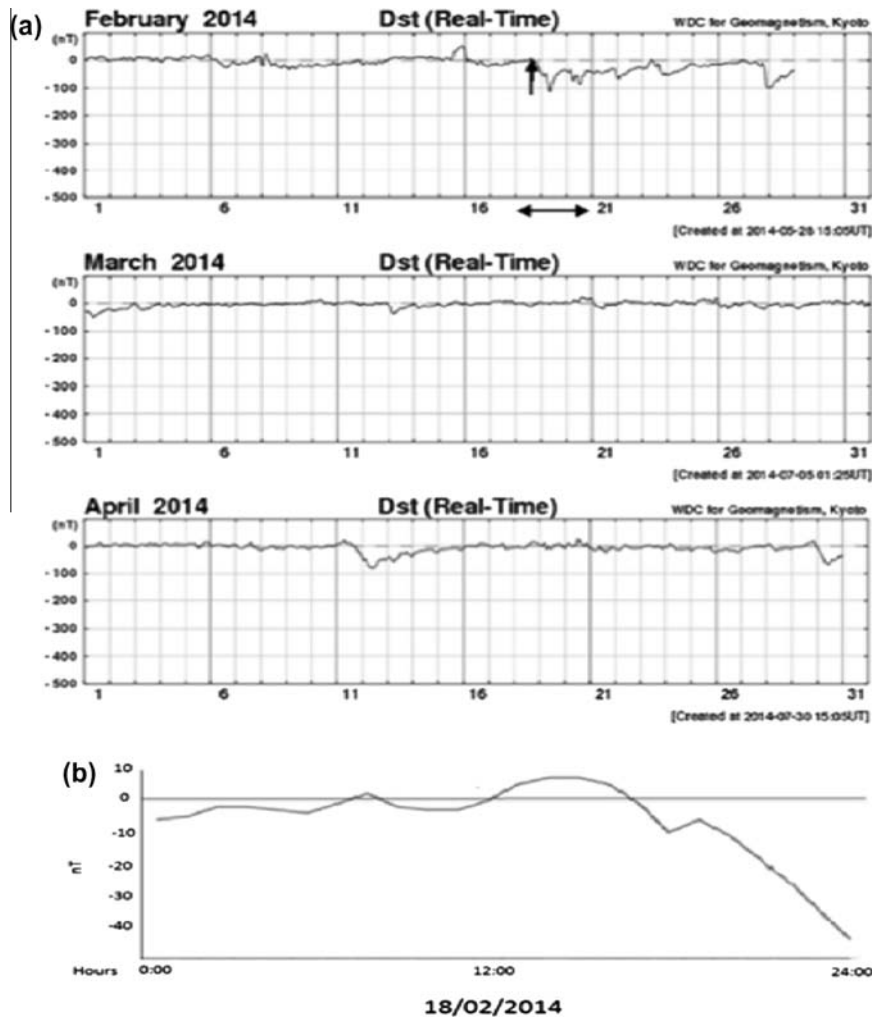


Fig. 2. Dst data from the WDC_Kyoto. (a) During the period from the 4th of February to the 10th of April, 2014. (b) GS-SSC, 18th of February 2014.

Table 1
The 15 geomagnetic storms with $Dst < -100$ nT from 1996 to 2008.

| Event/category | GS level by NOAA | Date | UT |
|----------------|------------------|------------|-------|
| GS_1/strong | G3 | 30/08/2004 | 23:00 |
| GS_2/great | G4 | 20/11/2003 | 22:00 |
| GS_3/strong | G3 | 31/08/2005 | 20:00 |
| GS_4/strong | G4 | 08/05/2005 | 19:00 |
| GS_5/strong | G3 | 18/08/2003 | 16:00 |
| GS_6/strong | G3 | 22/01/2004 | 14:00 |
| GS_7/strong | G5 | 27/07/2004 | 14:00 |
| GS_8/strong | G4 | 30/05/2005 | 14:00 |
| GS_9/strong | G2 | 04/04/2004 | 13:00 |
| GS_10/strong | G4 | 11/09/2005 | 11:00 |
| GS_11/strong | G2 | 18/06/2003 | 10:00 |
| GS_12/strong | G3 | 18/01/2005 | 9:00 |
| GS_13/strong | G4 | 15/05/2005 | 9:00 |
| GS_14/strong | G5 | 08/11/2004 | 7:00 |
| GS_15/strong | G3 | 12/07/2003 | 6:00 |

NOAA: National Oceanic and Atmospheric Administration (http://www.swpc.noaa.gov/NOAA_scales).

3. Facilities and equipment

Here we describe the equipment we used to perform the experiments and the handling of the animals. The facilities are at the School of Sciences at the University of Mexico, (Universidad Nacional Autónoma de México (UNAM)).

3.1. The semianechoic chamber (SC)

To screen ambient magnetic fields up to 10^{-8} T we used a SC localized in the School of Sciences of the UNAM (latitude 19.32° N, longitude -99.18° W), which is relatively close to the TEO observatory. The SC model is the FACT 3 EMC Test Chamber by ETS-LINDGREEN and ESCO Technologies Company Project Number: AP577. The dimensions of the SC are $7.62 \text{ m} \times 5.18 \text{ m} \times 5.64 \text{ m}$, and it uses an hybrid absorber of ferrite tiles that allows a screening of ± 4.0 dB, that is equivalent to a magnetic field of 5 nT [http://www.compeng.com.au/emc_conversion_tables_field_strength_calculator.aspx].

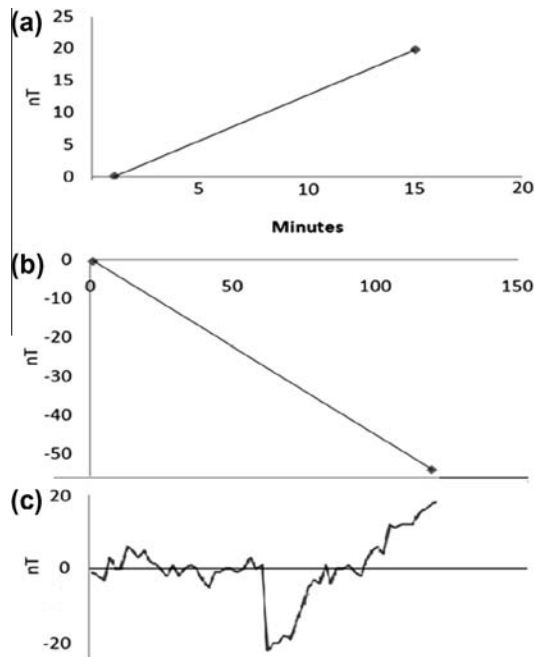


Fig. 3. (a) eSSC profile constructed with the averages of the SSC phase of 15 GS observed between 1996 and 2008 with $Dst \leq -100$ nT. (b) ePF profile constructed with the averages of the minimum Dst values of the PF phase of 15 GS observed between 1996 and 2008 with $Dst \leq -100$ nT. (c) Profile_GS, constructed with the H observed measurements of TEO observatory during the SSC day of the GS the 18th of February 2014 during 14:00–16:00 LT.

3.2. Animal handling

The ten Wistar male rats used in the experiment were raised in the biotherium of the School of Science. The weaning took place 30 days after they were born, when they were handed over to the researchers. As the experiment had to be carried out during the day due to use restrictions of the SC, and because it is during the dark periods when the rats are active, we had to alter their circadian cycle. We changed the circadian cycle by lagging it by 6 h. From the moment that the researchers received the rats, they were subjected to a photoperiod of 12 h of light and 12 h of darkness, initiating dark periods at midday and ending them at midnight of the same day. To achieve this, we used artificial illumination with a LED lamp (FRELUX 10 W 900 lm). The rats were placed in standard cages of transparent acrylic measuring 22 cm wide, 43.5 cm long and 20 cm high, covered with a material that allowed air circulation. The same temperature (23 °C), humidity (50–60%) and cleaning time were preserved since they were born. They were fed with a suitable meal for small rodents (50001 Purina) and *ad-libitum* water and food.

To diminish the stress of the animals we always transported the rats from the biotherium to the SC one hour before the experiments started, in cages that did not allow light to enter. They were constantly and gently handled while measuring their arterial pressure (AP) as part of their daily routine. Also, the same researcher, who never used

any kind of perfume in her person or cloths, performed the handling and cleaning of the animals.

3.3. Arterial pressure (AP) monitor

The monitor was developed in the Department of Instrumentation of the National Institute of Cardiology in México (Instituto Nacional de Cardiología Ignacio Chávez). It allowed us to measure the AP in a non-invasive way, minimizing the stress on the rats. Each signal after amplification and filtering went to a digital–analog converter and afterwards to a computer that processed, stored and displayed the data. Further details are found in (Flores-Chávez et al., 2002, 2007).

3.4. The simulator

We constructed a device to reproduce specific magnetic field variations, named the simulator, which is formed by a coil inductor and a microcontroller. The coil inductor reproduced the magnetic field variation, and it has a set of coils made of a copper cable with a diameter of 0.5 mm and a longitude of 1.20 m. The coil inductor has a resistance of $214.4 \pm 0.0005 \Omega$, enduring a maximum charge of 0.80 A. The microcontroller provided the needed currents. It is a 12 bits digital–analog converter (ARDUINO DUE), which was programmed to reproduce the desired magnetic variations.

4. Experimental procedures

All the arterial pressure (AP) measurements passed the Lilliefors test, showing a normal tendency and therefore indicating that the t -Student test could be applied to these measurements. We also obtained the standard deviations (SD) and standard errors (SDE) associated to the t -Student test.

We performed the experiments with ten one-month-old normotense male Wistar rats. The experimental period was from the 4th of February 2014 to the 10th of April 2014, on Thursdays and Fridays from 14 to 16 h. The days of the week and time-lapses of experimentation were those that the SC managers allowed us.

4.1. The stimulations

Because of the SC galvanized steel cover, the geomagnetic field suffered a small deviation inside the SC, so in its interior we marked the geomagnetic north with a compass, aligning the cages in that direction. The stimulations were always carried out inside the SC. The SC was kept at a temperature of 23 °C. During the stimulation the SC remained totally dark.

The simulator was placed in the North–South direction, with the animal cage in its interior covered by a lid of dielectric material that allowed airflow. The microcontroller was outside the SC in a special place so as not to

interfere with the proper SC functioning. Previous to the experiment sessions, the simulator was programmed with the appropriate magnetic field variations: eSSC, eFP and Profile_GS described in Section 2.2.

4.2. Procedures

The rat AP was always measured immediately on their exit of the SC, and at the same time each day. The details of the AP measurement are the following (Flores-Chávez et al., 2002, 2007).

The experimental procedures were as follows:

Once the simulator was programmed, we placed a cage with 5 rats inside it. We powered in the microcontroller and started the stimulation with the SC closed. Once the stimulation ended, the microcontroller stopped automatically. Then we proceeded to measure the rats AP. Afterwards they were returned to the biotherium in the same manner they were carried to the SC. Once there, they were cleaned and given fresh food and water. Concerning the control subjects, we carried out the same experimental procedure described inside the SC, but without the magnetic field stimulation. We did this in three occasions to assess possible alterations of the rats AP due to the experimental process itself: the first time at the beginning of the experimental period (IC) from the 4th to the 6th of February, the second time at the middle of the experiment (InC) the 11th of March, and the third time almost at the end of the experiment (FC) the 9th of April, always checking that no GS were in course.

To stimulate the animals with different types of magnetic field variations, we applied the profile eSSC for 15 min and the profiles eFP and Profile_GS for 2 h. The day of the GS (18th February, 2014) we followed the same procedures stated above without the magnetic field stimulations in order to measure the AP changes due to the GS and we continued these procedures on the 18th, 19th, 20th, 25th, 26th, and 27th of February, when geomagnetic activity diminished.

5. Results

The Dst behavior during the experimental period is observed in Fig. 2. During the GS marked with a circle in the figure, we had an experiment going on. The GS started the 18th of February with a SSC, reaching the Dst minimum on the 19th at 9:00 UT with a $Dst = -112$ nT and presented a decrease of activity up to the 27th of February.

5.1. Experiments with magnetic field stimulations

The simulator used three data sets described in Section 2.1, each reproducing a certain type of geomagnetic field variation. The first variation profile was constructed

with the averages of the Dst SSC-phase of the 15 GS (see Table 1) using the SYM-H base. This variation has duration of 15 min and the simulated geomagnetic field increases from 0 to 19.76 nT. The latter value represents the 0.07% of the value of H (27611.9 nT), corresponding to the month of the GS occurrence during the experimental period; this is the eSSC profile (see Fig. 3a). The second profile is the average of the Dst PF of the 15 GS. It has duration of 2 h and the simulated geomagnetic field decreases from 0 to -54.19 nT, presenting a decrease of 0.19% of H , which is the eFP profile (see Fig. 3b). Both profiles have a linear behavior. The third type, Profile_GS, was constructed using the actual measurements of the geomagnetic field obtained from the TEO observatory during the SSC-phase of the GS (subtracting the base value of 27611.9 nT). It is clear that this third variation has a non-linear behavior, as it can be seen in Fig. 3c.

We needed control values in order to use the statistical tests mentioned in Section 4. The control values are the AP measurements of each rat before it was stimulated and during times of no GS. As mentioned in Section 4.2, we obtained three controls; Table 2 shows their values: initial (IC), intermediate (InC) and final (FC). The first column presents the number of AP measurements, the second, third and fourth columns the average, minima and maxima control values respectively, the two last columns are their standard deviation (SD) and associated error (SDE).

We simulated the eSSC and PF profiles. For the eSSC profile we found AP values shown in Table 3; in this type of stimulation we used the InC as a reference value when applying the t -Student test. Fig. 4a presents the comparison between the AP values and their control InC. Following the eSSC stimulation; we stimulated the animals with the FP profile with control value FC. We found the AP values of Table 4 and the comparison between the AP values and their control FC in Fig. 4b.

5.2. Experiments during the GS occurrence

During our experiments a GS occurred on the 18th of February with a SSC at 14:00 UT. Its principal phase occurred the 19th of February, and the minimum was reached at 09:00 UT with a $Dst = -112$ nT. The recovery phase occurred between the 19th and the 27th of February, presenting certain geomagnetic activity (see Fig. 5). In the upper part of the figure we presented the Dst during the GS days and in the lower part the daily AP values obtained. We measured the AP on the 18th, 19th and 20th as well as on the 25th, 26th and 27th of February. During the GS we obtained two data series, each one of 90 data. The first series corresponded to the subjects that were placed inside the SC, while second series corresponded to the subjects that remained outside the SC. In both cases, the AP measurements were taken at the same

Table 2

Control AP measurements: the animals were their own controls. They were not subjected to magnetic field stimulations and no GS was under way.

| | <i>N</i> | Average (mmHg) | Minimum (mmHg) | Maximum (mmHg) | SD | SDE |
|----------------------------|----------|----------------|----------------|----------------|------|------|
| Initial control (IC) | 50 | 116.50 | 107 | 127 | 5.02 | 0.71 |
| Intermediate control (InC) | 28 | 116.67 | 104 | 126 | 5.09 | 1.12 |
| Final control (FC) | 45 | 119.62 | 107 | 128 | 4.5 | 0.67 |

N = number of data; SD = standard deviation; SDE = standard deviation error corresponding to the *t*-Student test.

Table 3

AP values and statistical parameters of the eSSC and eFP profiles.

| | Mean (mmHg) | SD | <i>N</i> | SDE | Reference (mmHg) | <i>P</i> |
|----|-------------|------|----------|------|------------------|----------|
| AP | 114.85 | 4.20 | 52 | 0.58 | InC 116.67 | 0.003 |
| AP | 122.50 | 5.8 | 44 | 0.88 | FC 119.62 | 0.002 |

SD = standard deviation; SDE = standard deviation error, corresponding to the *t*-Student test. AP reference values: InC = Initial Control; FC = final control.

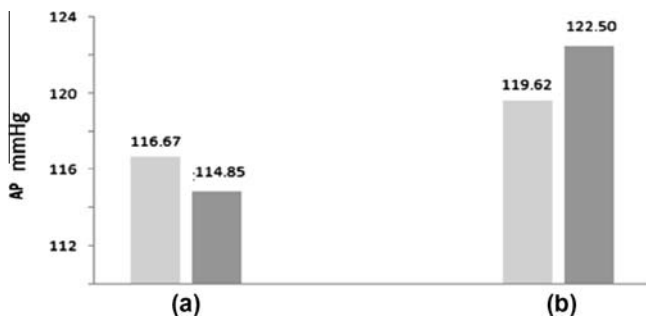


Fig. 4. AP measurements. (a) The first column shows the reference value InC = 116.67 mmHg and the second column the AP values after the stimulation with the eSSC profile. (b) The first column shows the reference value FC = 119.62 mmHg and the second column the AP values after the stimulation with the eFP profile. The standard deviations are too small to present them in the figure, but their values are in Table 3.

time of the day. Table 4 presents the AP average and their statistical parameters.

5.3. Reproducibility of results

As reproducibility is crucial to our experiment, we proceeded to reproduce the GS conditions of the 18th of February. According to Table 4, the largest AP changes occurred during the SSC day. We then prepared the simulator to reproduce it using the TEO geomagnetic field measurements taken during the GS, with a one-minute resolution, which we then used to prepare the Profile_GS of Fig. 3c. This profile was applied to the subjects for two hours inside the SC, always at the same time and sequence, on four non-consecutive occasions giving a total of 240 measurements. Fig. 6 shows the AP results.

The bars show the daily AP values for this experiment: in the first column we show the reference AP value InC, and the following columns show the four readings obtained. It is important to point out that in these days

the geomagnetic activity was low and without GS. Table 5 shows the corresponding statistical parameters.

6. Discussion

The experiments were carried out near the TEO geomagnetic observatory, in such a way that we monitored the local geomagnetic field. Geomagnetic variations that characterize GS are very small, of the order of 10^{-7} T, and can easily be camouflaged by the ambient magnetic fields. In order to screen the ambient fields we performed the experiments in a semianechoic chamber (SC) that is able to screen ambient magnetic fields of up to 10^{-8} T. We are confident that the simulated and natural magnetic variations were responsible for the animals AP changes.

The manipulation of the animals 30 days after they were born, allowed us to reduce their stress to a minimum. That is why the reference AP levels throughout the whole experimental period varied in less than 2.6%, while the AP changes attributed to the geomagnetic field variations were of $\sim 10\%$. We also had long periods of quiet geomagnetic activity that allowed us both to establish reference AP levels and apply the Profile_GS used in the reproducibility part of the experiment.

When we stimulated the subjects with a profile constructed from the average changes associated to the SSC and PF GS phases we did not observe statistically significant AP changes, but when we used the observed GS Profile_GS constructed from the actual measurements of the SSC GS phase, we did find statistically significant AP changes. We propose that this behavior was due to the linear (based on averages) and non-linear (based on measurements) character of the constructed stimulations.

We also identified the GS phase that produced the largest AP variation: the SSC phase. We found that the geomagnetic field variations associated to this phase were capable of increasing the subjects AP between 7% and 9% with respect to the reference value. The intensity of the magnetic field associated to the SSC phase was 7 nT, while the intensity of the minimum during the PF was -112 nT. We noticed that the magnetic field changes are larger for the SSC than for the PF. For this reason, we propose that it is more important the variation of the geomagnetic field than its intensity. Therefore, our results coincide with those of Azcárate et al. (2012), Krylov et al. (2014) and Yu and Shang (2014) who also found that concerning arterial pressure variations, it is more important the variation of the geomagnetic field than its intensity.

Table 4
AP values and statistical parameters during the GS starting the 18th of February 2014.

| Date | Mean (mmHg) | % | SD | SDE | Reference value InC (mmHg) | P |
|------------------------------------|-------------|------|-------|------|----------------------------|----------|
| <i>AP during GS within the SC</i> | | | | | | |
| 18/02/2014 | 127.5 | 9.28 | 9.7 | 2.5 | 116.67 | 0.0007 |
| 19/02/2014 | 118.0 | 1.13 | 5.0 | 1.3 | 116.67 | 0.3 |
| 20/02/2014 | 118.9 | 1.91 | 5.9 | 1.5 | 116.67 | 0.17 |
| 25/02/2014 | 119.2 | 2.17 | 2.9 | 0.7 | 116.67 | 0.004 |
| 26/02/2014 | 121.0 | 3.71 | 4.4 | 1.1 | 116.67 | 0.002 |
| 27/02/2014 | 119.6 | 2.51 | 2.4 | 0.6 | 116.67 | 0.0003 |
| <i>AP during GS outside the SC</i> | | | | | | |
| 18/02/2014 | 125.2 | 7.31 | 11.08 | 2.9 | 116.67 | 0.009 |
| 19/02/2014 | 116.8 | 0.11 | 3.4 | 0.9 | 116.67 | 0.855 |
| 20/02/2014 | 118.3 | 1.40 | 6.3 | 1.69 | 116.67 | 0.338 |
| 25/02/2014 | 120.9 | 3.62 | 1.8 | 0.48 | 116.67 | 0.000001 |
| 26/02/2014 | 120.0 | 2.85 | 12.7 | 3.66 | 116.67 | 0.38 |
| 27/02/2014 | 118.4 | 1.48 | 3.0 | 0.8 | 116.67 | 0.052 |

SD = standard deviation; SDE = standard deviation error corresponding to the *t*-Student test.

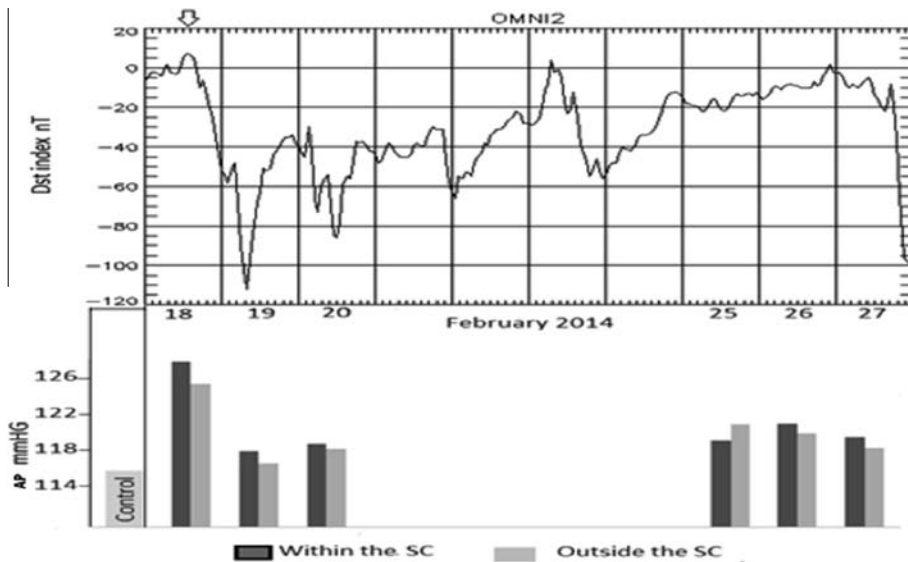


Fig. 5. The upper part shows the Dst behavior from the 18th to the 20th and from the 25th to the 27th of February, 2014. The lower part shows the daily AP values, the dark columns indicate the measurements inside the SC and the light columns those outside. The white columns show the AP control values. The standard deviations are too small to present them in the figure, but their values are in Table 4.

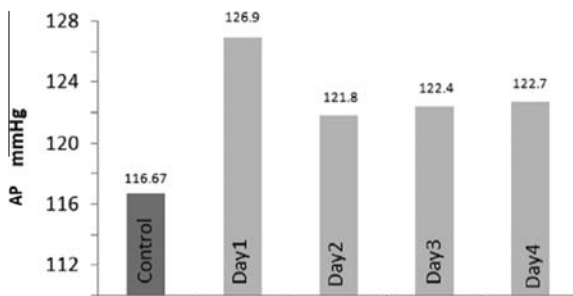


Fig. 6. The AP values obtained by the stimulation with the Profile_GS. The stimulations were carried out for four non-consecutive days. The first column shows the InC reference value. The standard deviations are too small to present them in the figure, but their values are in Table 5.

The day of the GS (18th of February), signaled by the SSC, presented the largest AP increase with the largest standard deviations (see Table 5), coinciding with the observation that the animals were particularly restless, showing a behavior not seen before: they were wet and covered with the material of the standard bed; also the container lid was covered with this material (see Fig. 7). The same behavior was found again the day of the stimulation with Profile_GS.

The ANOVA test applied to both groups shows that they are not independent, implying that their SD overlap; however the group inside the SC increased its AP in 9.3% with respect to the reference value (in this case the InC), the group outside the SC increased its AP in 7.3%,

Table 5
AP values and statistical parameters during the stimulation with the Profile_GS1.

| Repetitions | Mean (mmHg) | Increase (%) | SD | SDE | Reference value InC (mmHg) | P |
|-------------|-------------|--------------|------|------|----------------------------|----------|
| 1 day | 126.9 | 8.77 | 8.30 | 1.54 | 116.67 | 0.00000 |
| 2 day | 121.8 | 4.39 | 6.70 | 1.21 | 116.67 | 0.00027 |
| 3 day | 122.4 | 4.91 | 9.40 | 1.72 | 116.67 | 0.0024 |
| 4 day | 122.7 | 5.17 | 7.30 | 1.33 | 116.67 | 0.000095 |

SD = standard deviation; SDE = standard deviation error corresponding to the *t*-Student test.



Fig. 7. The cage where the animals were routinely introduced into the SC. Clearly the plastic lid shows the pieces of the standard bed material left by the rats during the GS starting the 18th of February, 2014.

compared to the same reference value InC (see Table 4). We interpret this as showing that the effects on the AP produced by the GS are more clearly perceptible within the SC, however they are also quantifiable outside the SC even if there are other electromagnetic environmental effects.

The ability to reproduce the AP variations was also another fundamental part of our experiments. As Fig. 6 shows, there was an increase in the AP of the rats when they were stimulated by the Profile_GS. The AP increase was smaller after repeated stimulations with the Profile_GS because of the adaptive process. Therefore, our results coincide with those of Dimitrova et al. (2004, 2009), who found arterial pressure increases under weak local geomagnetic changes and when major global geomagnetic storms took place during the day before a GS, a time including a SSC.

Since life started to develop on Earth, the geomagnetic field variations associated to the dawn (increases) and dusk (decreases) are similar in magnitude to the SSC variations (Shinbori et al., 2009; Tsuji et al., 2012). The physiological response of living beings to changes in the geomagnetic field is expected because they have been present from the onset of life on Earth. We propose that geomagnetic fields can function as a zeitgeber in circadian cycles, then geomagnetic rhythms may organize physiological rhythms (Wever, 1968; Funk et al., 2009).

The cell membrane is considered to be the cell's part that most likely interacts with a magnetic field, and the majority of the results point to an effect on the rate of ions or ligand

binding to, e.g. a receptor site acting as a modulator of signaling cascades often calcium/calmodulin-dependent processes, cAMP and growth factors. In most cases the magnetic effect is ascribed to Ca^{2+} ions. The biochemical reactivity of ions bound in the molecular clefts of macromolecules may be affected by magnetic fields via changes in the spatial orientation of movement or by changing Larmor precession frequencies. For fields in the nT range, the bound lifetime must be sufficiently longer than 1 ms (Muehsam and Pilla, 1998). Then these studies suggest the geomagnetic field may act on the membranes through the mechanisms described above.

7. Conclusions

We stimulated the subjects with a magnetic profile constructed from the average changes associated to the SSC and PF of a GS and found no statistically significant AP changes. However, during the GS that occurred during the experimental period and again when we used the observed GS variations to construct the actual measurements of the SSC day, we did find statistically significant AP changes.

We found that the geomagnetic field variations associated to SSC day of the GS were the largest and capable of increasing the subjects AP between 7% and 9% with respect to the reference value. Under these magnetic variations, the subjects presented a notably restless behavior not seen under other conditions.

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PRESENTACIÓN DEL ARTÍCULO: *"Artificial reproduction of magnetic fields produced by a natural geomagnetic storm increases systolic blood pressure in rats."*

Por último en este tercer artículo se hace una discusión detallada de los elementos de control en las ratas adultas tipo Wistar. Éstas fueron elegidas como modelo del sistema cardiovascular con la asistencia del MVZ Pedro L. Flores del Instituto Nacional de Cardiología Ignacio Chávez. Los animales provienen del Bioterio de la Facultad de Ciencias de la UNAM, así mismo nos dieron acceso a sus instalaciones para el hospedaje y cuidado, permaneciendo allí durante todo el proceso previo y durante la experimentación. Lo anterior fue decisivo para mantener a los animales en un ambiente confortable y sin grandes cambios. Dado que las ratas inician su actividad durante la noche, fue necesario condicionarlas en un ciclo de luz y oscuridad con un corrimiento de 6 horas, mantenerlas alejadas de líneas de corriente y con iluminación tipo LED y tenue roja del mismo tipo para manejo en la oscuridad en los procedimientos experimentales.

La Cámara Semianecoica se encuentra en las mismas instalaciones de la Facultad de Ciencias, no obstante su cercanía se trasladaron a los animales una hora antes de los procedimientos experimentales, para que las incomodidades del traslado no afectaran los resultados, en cámaras oscuras para preservar el corrimiento que se realizó desde su destete hasta el final de los procedimientos experimentales. Durante una semana se llevaron a cabo todos los procedimientos sin estimulación de campos magnéticos a una temperatura de 23°C hasta que las lecturas de la PA en las ratas se estabilizó. Todos los procedimientos se llevaron a cabo con puntualidad estricta.

La tesista realizó toda la manipulación de las ratas con gentileza logrado en ellas un estado de comodidad y confianza, también dejó de utilizar cualquier tipo de perfume para

no perturbarlas. La regularidad lograda en los procedimientos permitió que al momento en que ocurrió una TG, todos los mecanismos de control de variables estuvieran actuando y por tanto se pudieron hacer mediciones confiables de la PA de las ratas. Posteriormente se pudo simular la variación observada en TEO del campo geomagnético durante la TG. Llegamos a la conclusión de que los cambios en el campo geomagnético asociados con una tormenta geomagnética en su primera día pueden producir una respuesta fisiológica medible y reproducible en PA.

Artificial reproduction of magnetic fields produced by a natural geomagnetic storm increases systolic blood pressure in rats

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Abstract The incidence of geomagnetic storms may be associated with changes in circulatory physiology. The way in which the natural variations of the geomagnetic field due to solar activity affects the blood pressure are poorly understood and require further study in controlled experimental designs in animal models. In the present study, we tested whether the systolic arterial pressure (AP) in adult rats is affected by simulated magnetic fields resembling the natural changes of a geomagnetic storm. We exposed adult rats to a linear magnetic profile that simulates the average changes associated to some well-known geomagnetic storm phases: the sudden commencement and principal phase. Magnetic stimulus was provided by a coil inductor and regulated by a microcontroller. The experiments were conducted in the electromagnetically isolated environment of a semi-anechoic chamber. After exposure, AP was determined with a non-invasive method through the pulse on the rat's tail. Animals were used as their own control. Our results indicate that there was no statistically significant effect in AP when the artificial profile was applied, neither in the sudden commencement nor in the principal phases. However, during the experimental period, a natural

geomagnetic storm occurred, and we did observe statistically significant AP increase during the sudden commencement phase. Furthermore, when this storm phase was artificially replicated with a non-linear profile, we noticed a 7 to 9 % increase of the rats' AP in relation to a reference value. We suggested that the changes in the geomagnetic field associated with a geomagnetic storm in its first day could produce a measurable and reproducible physiological response in AP.

Introduction

Geomagnetic storms affect space-borne or ground-based technological systems and may have effects on biological regulation. In particular, changes in the cardiovascular systems of humans and other mammals have been recently correlated with the presence of geomagnetic storms (Vencloviene et al. 2013). GS are initiated when energy is transferred from the solar wind into the Earth's magnetosphere, a process that appears to be controlled by the rate of magnetic reconnection

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between the southward component of the interplanetary magnetic field and magnetosphere fields (Dungey 1961; Russell and Elphic 1979). Such storms lead to the intensification of the ring current enhancement of the trapped magnetospheres' particles population (Gonzalez et al. 1994). Ring current particles are energized during a GS, producing a decrease in the geomagnetic disturbance storm index measured in nanoteslas (nT), which measures the development of the ring current. Dst is frequently used as an indication of the relative strengths of GS (Gonzalez et al. 1994). GS are categorized as: (i) weak ($Dst < -30$ nT), (ii) moderate ($Dst < -50$ nT), (iii) strong ($Dst < -100$ nT), (iv) severe ($Dst < -200$ nT), and (v) great ($Dst < -350$ nT) (Olawepo and Adeniyi 2014). It has been observed that the Dst typically consists of a three-phase pattern: (1) a sudden storm commencement (SSC) followed by (2) a period of fast decay or principal phase (PP) and, finally, (3) a recovery phase (RP), where the Dst gradually returns to its quiet time value. Although this is the canonical behavior, the SSC may not be always present (Takahashi et al. 1991; Feldstein et al. 2000; Spencer et al. 2011).

Life on Earth has evolved in the presence of natural magnetic fields, and biological systems may respond to them in diverse ways (Maffei 2014; Okano 2008a, b; Lohmann 2010; Johnsen and Lohmann 2008; Ulmer et al. 2002; Brocklehurst and Mc Lauchlan 1996). It is possible that some of the physiological mechanisms of control in mammals are sensible too. In particular, associations between GS through the use of geomagnetic indices and physiological responses, such as variations in AP or higher incidence of death due to heart illnesses, have been reported (Okano 2008a; Dimitrova et al. 2004; Khabarova and Dimitrova 2008; Mendoza and Sanchez de la Peña 2010; Kuz'mina et al. 2014). Also, diverse reports correlate geomagnetic activity with the cardiovascular physiology, using different geomagnetic indices (e.g. Dimitrova et al. 2008; Shaposhnikov et al. 2014; Vencloviene et al. 2013; Mavromichalaki et al. 2012; Papailiou et al. 2011; Gurfinkel et al. 2012). There are reports in which a magnetic field affects microcirculation (Brix et al. 2008; Morris and Skalak 2005) and AP (Ghione et al. 2004; Okano 2008a, Gmitrov 2007). Static or weak intensity magnetic fields modify the microcirculation through the arterial baroreflex initiated by receptors in the aortic arch that causes changes of peripheral vasomotor tone (Okano 2008a).

In order to better understand the effect of the geomagnetic field natural variations on animal physiology, it is necessary to separate them from the artificial electromagnetic field variations and to reproduce the GS phases to identify which of them may cause the largest physiological effects. In the present study, we tested whether the (AP) is affected in rats of 30 days of age at the beginning of the study by simulated changes in the geomagnetic field observed during a GS. The experiments were carried out in an isolated environment inside a semi-anechoic chamber where environmental electromagnetic effects were shielded.

Methods

Indices of geomagnetic storms

The Dst index was constructed according to description in the IAGA Bulletin No 40, (<http://wdc.kugi.kyoto-u.ac.jp/dst/dir/dst2/onDstindex.html>), with the hourly average measurements obtained from four observatories distributed in low and middle latitude locations. Also, the SYM-H index (1-min resolution) was constructed; both indices measure the symmetric portion of the magnetic field horizontal component (H) near the magnetic equator (Wanliss and Showalter 2006).

To assess the GS behavior, we used the Dst and SYM-H indices, as well as the H data. The indices can be found in the Space Physics Data Facility OMNIWeb (<http://omniweb.gsfc.nasa.gov/form/dx1.html>) and the WDC_Kyoto (<http://wdc.kugi.kyoto-u.ac.jp/index.html>). The H data were obtained from the Teoloyucan Magnetic Observatory (TEO) (<http://geomaglinux.geofisica.unam.mx>), and INTERMAGNET network (<http://www.intermagnet.org/data-donnee/download-eng.php>) respectively. TEO observatory is located at the following geographic coordinates: latitude 19.75° N, longitude -99.17° West.

Observation and stimulation periods, construction of magnetic field variations

We used the geomagnetic activity data of two periods: January 1996 to December 2008 and February 4 to April 10, 2014. Figure 1 shows the Dst behavior of the WDC_Kyoto data during the latter period. The oval in the upper panel indicates a natural GS (February 18th to 27th); the black arrow shows the day when the SSC phase occurs; gray bars indicate the days of animal test inside the semi-anechoic chamber. Therefore, this study consists of data obtained both in quiet natural geomagnetic environment and during a natural GS.

In order to simulate a GS profile, we searched for strong GS ($Dst < -100$ nT) in the databases previously indicated, from 1996 to 2008, and found 15 GS. Data were analyzed through a MatLab program. We obtained the SSC and PP phase averages from these 15 GS and constructed two lineal profiles labeled eSSC (Fig. 2a, left panel) and ePP (Fig. 2b, left panel). While the study was performed, a natural GS occurred, with $Dst < -100$ nT (indicated with an oval in Fig. 1). It did not present a SSC as such, but a gradual Dst increase on February 18, 2014; the PP minimum was on February 19, 2014 at 9:00 UT with $Dst = -112$ nT, and the RP persisted up to February 27, 2014. We used the data of February 18 (MLT 14:00 to 16:00, local time, issued by TEO), subtracting the H average of that day ($H = 27,611.9$ nT), to obtain the anomalies and to construct a profile named "Profile_GS" shown in Fig. 4a (left panel).

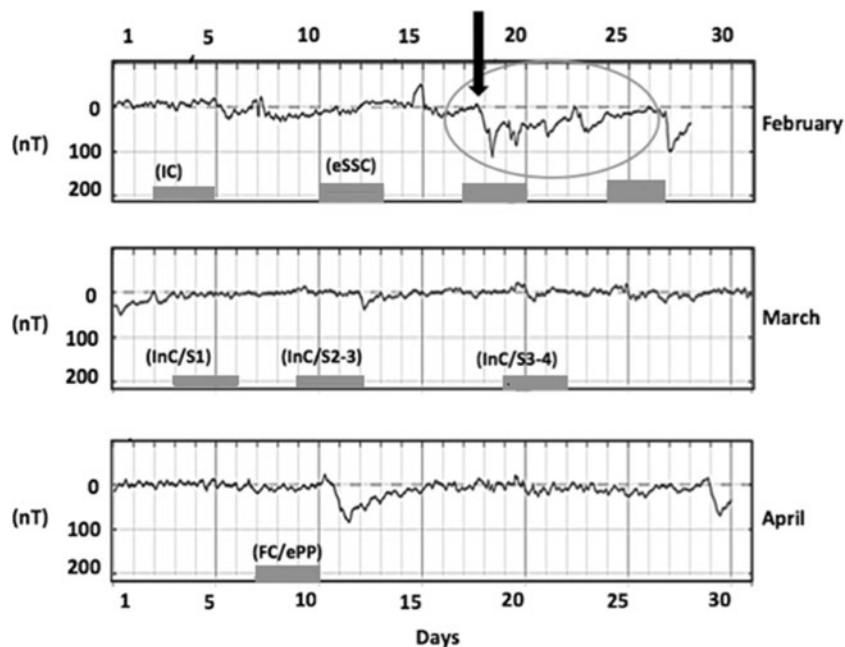


Fig. 1 Dst data from the WDC_Kyoto observed during the experiment. The time period from the 4th of February to the 10th of April 2014 includes a natural GS recorded at February 18th to 27th indicated with an oval. The arrow shows the beginning of the GS. The gray bars at the bottom indicate the days of the experiment. From left to right: top panel, the first bar, February 3–5, corresponds to the initial control (IC) measurements; the second bar, February 11–13, corresponds to the

eSSC stimulus (eSSC); the third and fourth bars, February 18–20 and 25–27, correspond to measurements during the GS. Middle panel, the first, second and third bars, March 4–6, 10–12 and 19–20, correspond to InC and Profile_GS in four occasions S1, S2, S2, and S4. Bottom panel, the first bar, April 8–10, corresponds to FC and stimulation of the main phase ePP (FC/ePP)

Facilities and equipment

The semi-anechoic chamber

All the experiments were performed at the facilities of the Facultad de Ciencias at the Universidad Nacional Autónoma

de México (UNAM); latitude 19.32° N, longitude –99.18° West. In order to filter the ambient magnetic fields up to 10^{-8} T, we used a semi-anechoic chamber (SC) (FACT 3 EMC Test Chamber by ETS-LINDGREEN and ESCO Technologies Company Project Number AP577), with dimensions 7.62 m × 5.18 m × 5.64 m. It uses a hybrid absorber of ferrite tiles that allows a deviation from normal attenuation (NSA) for 1kHz exceeds 40–60 dB. [http://www.compeng.com.au/emc_conversion_tables_field_strength_calculator.aspx].

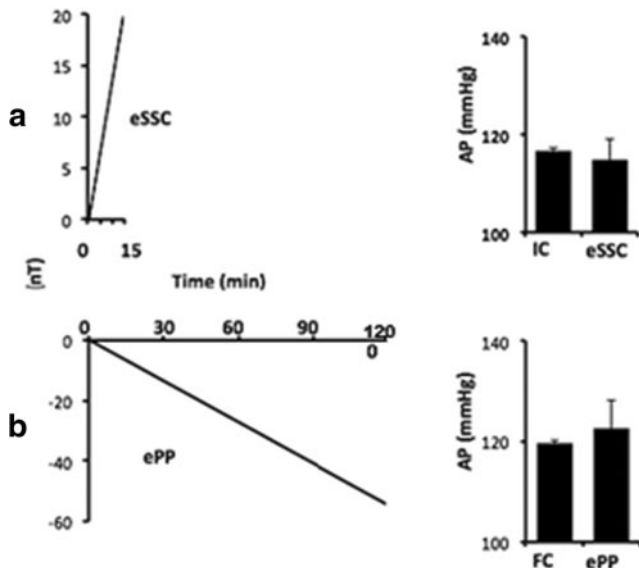


Fig. 2 a Linear variations of the eSSC profile and AP variations under the eSSC profile compared to the IC value. b Linear variations of the ePP profile and AP variations under the ePP profile compared to the FC value

Animal handling

Ten Wistar male rats were raised at the Facultad de Ciencias vivarium under artificial light-dark cycles 6 h advanced (photophase, 0:00–12:00 h; LED lamp FRELUX, 10 W, 900 lm), environmental temperature of 22–25 °C; tap water and regular rodent chow (Purina©, 50001) were provided ad libitum. After weaning, the rats received gentle handling on a regular basis in order to reduce stress at the time of AP determinations.

Rats were handled in the dark phase under a dim red light environment between 13:00 and 18:00 h when they were active. Two groups of five rats were placed in transparent acrylic cages (22 cm wide, 43.5 cm long and 20 cm high) at the same temperature (23 °C) and humidity (50 to 60 %). Cleaning and

handling were always done at the same time of the day, as well as all the AP measurements. The rats were transferred to the SC in darkness at least 1 h before exposure to simulated magnetic fields and then AP was tested. The same person always handled all the animals.

Arterial pressure measurement

We performed the test in rats between February 4 and April 10, 2014. AP was determined with a non-invasive instrument designed at the Department of Instrumentation of the Instituto Nacional de Cardiología (National Institute of Cardiology of Mexico) (Flores-Chavez et al. 2002, Flores-Chavez et al. 2007). The procedure was the following: The rat's tail was inserted into a latex chamber contained in a 13-mm-diameter rigid tube. The latex chamber was coupled to a pressure transducer connected to a direct current amplifier, and lower and upper filters 0.5 to 300 Hz were used to obtain the signal of pulses caused by the blood passage through the rat's tail. The chamber was inflated until the pulse in tail's circulation stopped. Then, the pressure in the chamber was gradually released until the pulse was detected. Signal amplification was processed and stored in a personal computer until further analysis. In order to minimize the stress during AP measurements, the animals were gently handled in a warm and comfortable position during the procedure. In each assay, a cage covered by a lid of dielectric material and containing a group of 5 rats was placed inside the coil, then a magnetic field was induced or not (controls) in the protocols indicated below. The AP was measured in all tested animals inside the SC without artificial magnetic stimulation and in quiet geomagnetic conditions; this is referred as controls. All the rats were used as their own control. We measured the controls in three different occasions, which are labeled as initial control (IC), 3 to 5 of February, intermediate control (InC), 4–6, 10–12 and 19–20 of March, and final control (FC), 8 of April.

One week after the IC measurements, between February 11–13 (see Fig. 1), all the animals were exposed to an artificial stimulus corresponding to a SSC phase indicated as eSSC. Then, the animals were returned to the vivarium until further tests. When the natural GS was detected (Fig. 1, February 18), the AP of five rats was measured inside the SC (with no artificial stimulation), while other five rats were measured outside. After this procedure, we compared the AP measurements with the IC values. We performed AP measurements during February 18, 19, 20, 25–27, all within the development of the GS. During March 4–6, 10–12 and 14–21, the simulated magnetic profile shown in Fig. 4 and the intermediate control (InC) were used alternately in rats, in such a way that all the ten animals were tested with four repetitions in each condition (S1, S2, S3, S4 in Fig. 4). Finally, during April 8–10, the rats were tested with and without the stimulus corresponding to

the artificial principal phase (ePP) inside the SC and compared to the FC.

Data analysis

The AP data series were reviewed with the Lilliefors test for normal distribution and then compared with a paired Student *t*-test to detect significant differences between groups (*p* value < 0.05 was considered statistically significant).

The magnetic field simulator and stimulations

We built a simulator of 1.20 m long and 0.5 m in diameter that reproduced specific magnetic field variations. The coil inductor was made of a copper cable (0.5 mm diameter, $214.4 \pm 0.0005 \Omega$, maximum current of 0.80 A) coupled to a microcontroller that provided the needed currents and programmed with the software provided by the seller (12 bits digital-analog converter, ARDUINO DUE) to reproduce the desired magnetic variations. The magnetic stimulations were always developed at 23 °C in darkness conditions. The geomagnetic field was measured inside the SC to determine the north-south line, as well as its magnitude, in order to align the coil. The microcontroller was located outside the SC in a different isolated room in order to reduce interference with the proper stimulator. Prior to the experiment, the simulator was programmed with the corresponding magnetic field variations: The eSSC profile (15 min) where a simulated magnetic field increased from 0 to 19.76 nT, which represents the 0.07 % of the value of *H* (27,611.9 nT) (see Fig. 2a). The ePP profile (120 min), where the simulated magnetic field decreased from 0 to -54.19 nT, presented a decrease of 0.19 % of *H* as it is indicated in Fig. 2a, b (left panels) respectively. When a natural GS was detected in course (February 18 2014), we followed the same procedures stated above without the stimulations and then we measured the AP changes from the 18th to the 20th, and from the 25th to the 27th of February, when the geomagnetic activity diminished. Then, we artificially reproduced the initial 2 h of the GS with the Profile_GS (Fig. 3a) using the actual measurements of the geomagnetic field obtained from the TEO observatory (Fig. 4a).

Results

Stimulation with the eSSC or ePP profiles

Figure 2 shows the average (± 1 SD) of the AP measurements without (controls) or with stimulations. No significant changes were observed between initial and final controls (IC = 116.5 ± 0.71 ; FC = 119.62 ± 0.67 mmHg), neither after the eSSC (AP = 114.85 ± 4.20 mmHg) nor ePP (AP = 122.5 ± 5.8 mmHg) stimulations. However, a larger

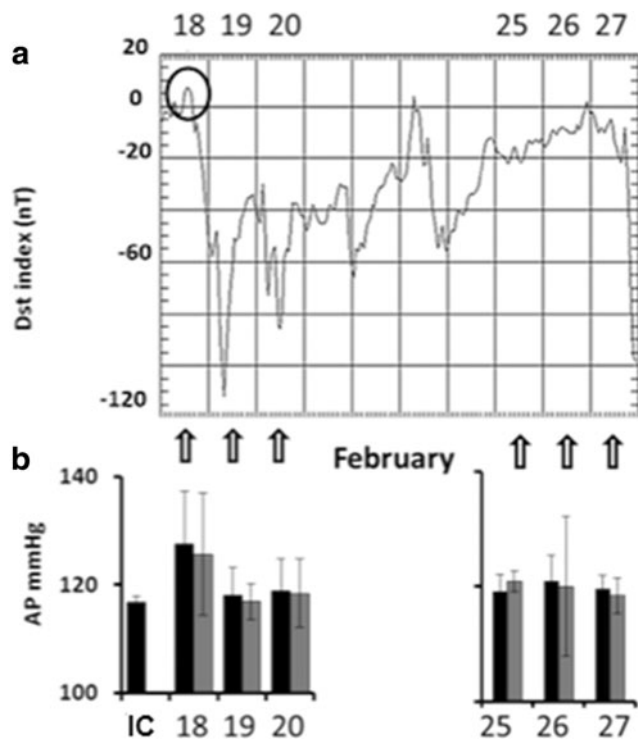


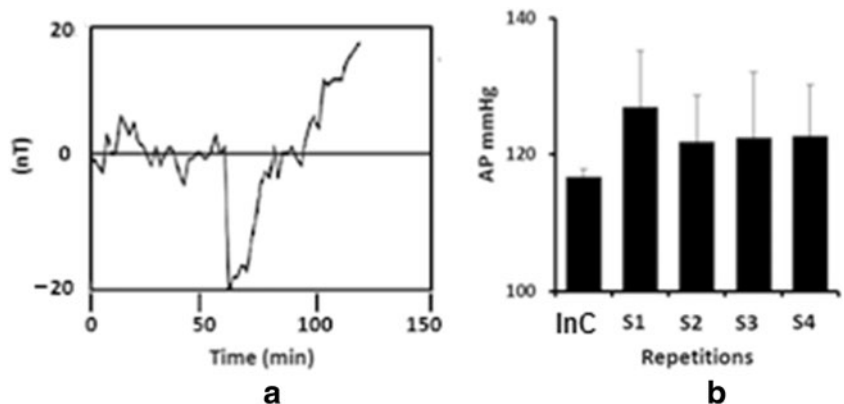
Fig. 3 **a** Dst behavior from the 18th to the 27th of February 2014. The circle indicates the geomagnetic storm variations used in the construction of the Profile_{GS}. **b** Average AP measurements obtained inside (black bars) or outside (gray bars) the semi-anechoic chamber. IC is the initial control value. Gray vertical bars are ± 1 standard deviation. The gray arrows indicate the correspondence to days in (a)

variability in response was observed after inducing the magnetic stimulation, seen in their standard deviations' amplitude.

Experiments during the GS occurrence

During the GS, which started on the 18th of February, the PP occurred on February 19th, and the minimum phase was reached at 09:00 UT with a Dst = -112 nT. The RP occurred between the 19th and 27th of February presenting intermittent geomagnetic activity (Fig. 3a). We measured the AP the 18th, 19th and 20th as well as the 25th, 26th and 27th of February;

Fig. 4 **a** GS simulation reproducing the profile obtained at February 18th. **b** Average AP variations after the stimulation. InC is the intermediate control day after day; consecutive stimulations are indicated as S1 to S4. Gray vertical bars are ± 1 standard deviation



the results are shown in Fig. 3b. The data obtained either inside (black bars) or outside the SC (gray bars) is plotted below. Figure 4a indicates the pattern used as a reference to be artificially reproduced in further tests. Table 1 presents the AP average and its statistical parameters.

Reproduction of GS effect on AP

In order to reproduce the effects observed on February 18th on AP, we exposed rats to an artificial magnetic field (profile shown in Fig. 4a), which corresponds to the Profile_{GS} in a 2-h period (single stimulation) indicated previously in Fig. 3a (circle inside the plot). We tested the animals' AP during four consecutive times with this profile, being aware that no geomagnetic storms were in course. A statistically significant increase in average AP (± 1 SD) was noted after the stimulation, as shown in Fig. 4b. Data shown in Table 2 indicate the percentage (%) of increase observed in relation to control conditions, (InC = 116.67 mmHg). We performed four stimulations with the Profile_{GS}. In the first stimulation, S1, the statistically significant increase passed the ANOVA test corresponding to the AP. The following three stimulations, S2, S3 and S4, did not pass the ANOVA test.

Discussion

Geomagnetic variations that characterize GS are considered small, about 10^{-7} T, and can easily be masked by the environmental magnetic fields. We performed the experiments in a semi-anechoic chamber that allowed us to screen ambient magnetic fields between 10^{-8} T to 10^{-6} T. Therefore, the simulated and natural magnetic variations were responsible (directly or indirectly) for animals' AP changes. All the experiments were carried out in a location near the TEO geomagnetic observatory; thus, the monitored geomagnetic field was similar to the one present at the experimentation facilities. Furthermore, the use of the semi-anechoic chamber ensures that the effects observed inside the chamber, of increase in AP,

Table 1 AP values and statistical parameters during the GS starting the 18th of February 2014

| Date | Mean mmHg | Number | Increase % | SD | SEM | Reference value IC (mmHg) | p value |
|-----------------------------|-----------|--------|------------|-------|------|---------------------------|----------|
| AP during GS within the SC | | | | | | | |
| 18 Feb 2014 | 127.5 | 30 | 9.28 | 9.7 | 2.5 | 116.50 | 0.0007 |
| 19 Feb 2014 | 118.0 | 30 | 1.13 | 5.0 | 1.3 | 116.50 | 0.3 |
| 20 Feb 2014 | 118.9 | 30 | 1.91 | 5.9 | 1.5 | 116.50 | 0.17 |
| 25 Feb 2014 | 119.2 | 30 | 2.17 | 2.9 | 0.7 | 116.50 | 0.004 |
| 26 Feb 2014 | 121.0 | 30 | 3.71 | 4.4 | 1.1 | 116.50 | 0.002 |
| 27 Feb 2014 | 119.6 | 30 | 2.51 | 2.4 | 0.6 | 116.50 | 0.0003 |
| AP during GS outside the SC | | | | | | | |
| 18 Feb 2014 | 125.2 | 30 | 7.31 | 11.08 | 2.9 | 116.50 | 0.009 |
| 19 Feb 2014 | 116.8 | 30 | 0.11 | 3.4 | 0.9 | 116.50 | 0.855 |
| 20 Feb 2014 | 118.3 | 30 | 1.40 | 6.3 | 1.69 | 116.50 | 0.338 |
| 25 Feb 2014 | 120.9 | 30 | 3.62 | 1.8 | 0.48 | 116.50 | 0.000001 |
| 26 Feb 2014 | 120.0 | 30 | 2.85 | 12.7 | 3.66 | 116.50 | 0.38 |
| 27 Feb 2014 | 118.4 | 30 | 1.48 | 3.0 | 0.8 | 116.50 | 0.052 |

were not produced by environmental magnetic fields within the limits of the chamber screening.

The rats used at the present study were handled on a regular basis during 30 days after they were born, which allowed us to reduce stress induced from manipulation; therefore, the control AP values along the whole experimental period varied in less than 2.6 %, compared with the AP changes attributed to the experimental magnetic variations of nearly 10 %. When the subjects were stimulated with a linear profile constructed from the average changes associated to the SSC and PP GS phases, we did not observe a statistically significant AP change (Fig. 2). However, a larger dispersion indicates more variability in the response. When we stimulated the rats with the Profile_GS (Fig. 4), we did find statistically significant AP increases.

However, we consider that the difference in results obtained in the tests (Figs. 2 and 4) might be due to the linear (based on averages, Fig. 2) and non-linear (based on measurements, Fig. 4) character of the constructed stimulations. It is possible that in our work, the main effects depend on the non-linear change of the magnetic stimulations, equivalent to those found at the beginning of the GS. The simulation of geomagnetic field variations associated to this moment (the intensity was

7 nT, while the intensity of the minimum during the PP was -112 nT) produced an increase of AP between 7 and 9 % respect to the control value (Fig. 4b) coincident with the previous reports by Dimitrova et al. (2004, 2008).

The local ionospheric electric currents induce magnetic fields that interact with those produced by the magnetosphere dynamics associated to the GS (Shinbori et al. 2009). We propose that the variation of the geomagnetic field is more important than its intensity. The first day of the GS (18th of February) presented the largest AP increase with the largest standard deviations (Fig. 3b), which is consistent with the observation that the animals were behaviorally stressed and found them wet and covered with the bedding material. The same behavior was observed again after the stimulation with Profile_GS, suggesting that the elevated AP might be indirectly caused by an uncomfortable situation for the animals and we cannot discard the option that the AP increase may be an indirect consequence of such behaviors, induced by biological systems different than those of the AP regulation; however, further research is required to confirm a change of behavior. It is important to point out that all other observations did not induce such behavior. The ANOVA test was valid only for the first stimulation (S1) and not for the subsequent; even

Table 2 AP values and statistical parameters during the stimulation with the Profile_GS

| Repetitions (day) | Mean (mmHg) | Number | Increase % | SD | SEM | Reference value InC (mmHg) | p value |
|-------------------|-------------|--------|------------|------|------|----------------------------|----------|
| 1 | 126.9 | 30 | 8.77 | 8.30 | 1.54 | 116.67 | 0.00000 |
| 2 | 121.8 | 30 | 4.39 | 6.70 | 1.21 | 116.67 | 0.00027 |
| 3 | 122.4 | 30 | 4.91 | 9.40 | 1.72 | 116.67 | 0.0024 |
| 4 | 122.7 | 30 | 5.17 | 7.30 | 1.33 | 116.67 | 0.000095 |

more, the AP increases were smaller for these simulations, and then we suggest that this behavior was due to adaptive processes. Also, we stress that the reproducibility in AP variations (Fig. 4) is a fundamental part of our experiments.

Since life started to develop on Earth, the geomagnetic field variations associated to dawn (increases) and dusk (decreases) are similar in magnitude to the SSC variations (Shinbori et al. 2009). The physiological response of living beings to changes in the geomagnetic field is possibly due to the fact that those fields have been present from the onset of life on Earth. Geomagnetic rhythms have been proposed as influential on physiological rhythms, (Wever 1970; Cremer-Bartels et al. 1984; Funk et al. 2009; Pilla 1974; Stoupel et al. 2011; Shaposhnikov et al. 2014; Yu and Shang 2014; Krylov et al. 2014). The cell membrane is considered to be the main target for magnetic field signals, particularly altering ions permeability or ligand binding, e.g., a receptor site acting as a modulator of signaling cascades often involving calcium/calmodulin-dependent processes, cAMP, and growth factors (Markov 2011). In most cases, the magnetic effect is ascribed to Ca^{2+} ions. The biochemical reactivity of ions bound in the molecular clefts of macromolecules may be affected by static magnetic fields via changes in the spatial orientation of movement or by changing Larmor precession frequencies. For fields in the nT range, the bound lifetime must be sufficiently longer than 1 ms (Muehsam and Pilla 1996). Other studies on the effect of weak magnetic field on biological systems indicate that variations on intensity and duration at the beginning of a GS may alter the dynamics at molecular level in spin transitions through mechanisms named iron-oxide-based magneto reception (Ritz et al. 2010).

Considering our results, we may conclude that stimulation with the average linear changes of a magnetic profile associated to the SSC and PP phases of a GS does not produce statistically significant AP changes. Also we provide evidence that natural GS may be associated to a significant increase in the AP of rats and that these effects may be reproduced by simulation of the Profile_GS. Further work is required to confirm these observations to better understand the mechanism that underlies such response and to correlate it with the observed influence of geomagnetic fields in other biological models.

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Compliance with ethical standards All procedures described in this article were carried out in accordance with the ethical guidelines of Declaration of Helsinki and National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023),

institutional guidelines, and the General Law of Health for Research Studies in Mexico (NOM-062-ZOO-1999).

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DISCUSIÓN Y CONCLUSIONES GENERALES:

Se realizó el experimento sin contratiempos y con una actividad geomagnética muy favorable que nos permitió completar ampliamente los objetivos planteados.

La meta más importante y complicada, era establecer un experimento en condiciones controladas en términos geomagnéticos, electromagnéticos y con un mínimo de estrés para las ratas, con las que se trabajó. Ello requería contar con un blindaje electromagnético que nos permitiera aislar el experimento de los campos electromagnéticos existentes en el medio. Esto lo logramos con el uso de la cámara semianecoica.

En lo referente a la estimulación con variaciones de campo magnético similares a las que se presentan en una TG, eran necesarias dos cosas: que la actividad geomagnética fuese mínima durante la estimulación y que durante esa baja actividad sucediera una TG con $Dst < -100nT$. La primera para poder estimular con la TG promedio calculada y la segunda, para estudiar un evento natural sin promedios, lo cual parecía un suceso improbable.

Al estimular con el promedio de una TG en sus fases de comienzo súbito y fase principal, en baja actividad geomagnética, no se registró un incremento en la PA significativo. Pero sucedió una TG con $Dst < -100nT$ y se pudo registrar un incremento en la PA sistólica en un 10%.

Con los registros de TEO en H, se reprodujeron en el simulador las variaciones del campo geomagnético asociadas a la TG y se estudiaron los efectos sobre la PA de las ratas.

Las variaciones lineales, asociadas a los promedios, de campo magnético no produjeron aumentos estadísticamente significativos en la PA, pero los no lineales sí lo hicieron.

La TG que sucedió durante el periodo experimental llega a su mínimo en Dst el 19 de febrero del 2014, le siguen varias TG con mínimos mayores, concluyendo el 25 de febrero. Notamos que el mayor incremento de PA se presenta en el día en que se inicio la TG, estando nuestro país del lado día, por lo que suponemos que las variaciones en la H se encontraban fuertemente influenciadas por corrientes ionosfericas, consecuencia de la incidencia del evento solar causante de la TG.

Con estos resultados podemos concluir que:

1. Los estímulos lineales con tasas de incremento del campo magnético del tipo de SSC y FP, no produjeron incremento en la PA, sin embargo las que no tienen un comportamiento lineal sí lo hacen.
2. La correlación de índices geomagnéticos tiene un error intrínseco al no considerar las variaciones locales del campo por la probable acción de sistemas de corriente ionosfericas.
3. La reproducibilidad de los efectos mostrando una disminución ante la repetición del estímulo nos habla de posibles procesos de condicionamiento.
4. Poner atención en la variabilidad ionosferica al suceder una TG parece ser el punto focal para continuar con la investigación.

Pensamos que los resultados obtenidos fueron satisfactorios debido a los estrictos niveles de control logrados, así como por la capacidad de reproducirlos.

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